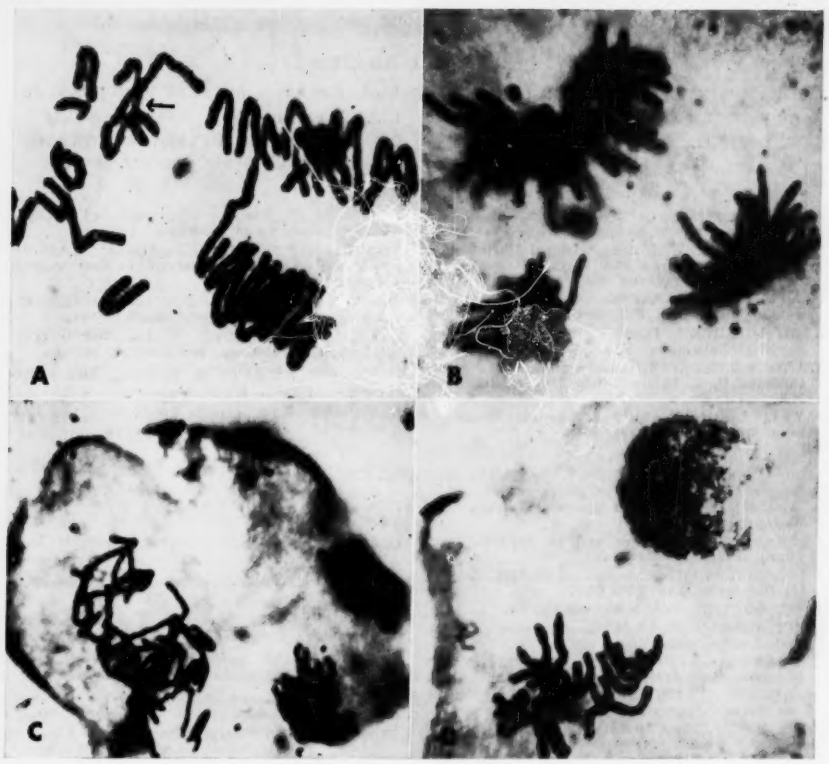
The Journal of HEREDITY



GENETIC CHANGES INDUCED BY VEGETABLE OILS
(See Page 177)

Multi-Ovary in Barley

Clubfoot in the House Mouse

Macromutation in Yellow Lupine

Blood-pH Differences in Inbred Mice

VOLUME 50 JULY-AUGUST, 1959 NUMBER 4

The American Genetic Association

An incorporated organization devoted to promoting a knowledge of the laws of heredity and their application to the improvement of plants, animals, and human racial stocks

SAMUEL L. EMSWELLER, President — Vice-President, H. J. MULLER

Honorary Vice-President, W. E. CASTLE

W. RALPH SINGLETON, Secretary — Treasurer, LAWRENCE G. BURK

ROBERT C. COOK, Editor — Managing Editor, BARBARA LAKE

EDITORIAL BOARD

J. T. BALDWIN, JR. — SHELDON C. REED — EARL L. GREEN — DELBERT T. MORGAN, JR.

GEORGE M. DARROW — JAY L. LUSH — HERBERT P. RILEY — JACK SCHULTZ

G. W. BEADLE — WALTON C. GREGORY — F. B. HUTT — G. LEDYARD STEBBINS

COUNCIL

RONALD BAMFORD, Dean of the Graduate School, University of Maryland, College Park, Md.

SAMUEL L. EMSWELLER, Principal Horticulturist, Division of Fruit and Vegetable Crops and Diseases, Plant Industry Station, Beltsville, Md.

BENTLEY H. GLASS, Professor of Biology, Johns Hopkins University, Baltimore, Maryland.

EARL L. GREEN, Director, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

WALTER E. HESTON, Geneticist, National Cancer Institute, U. S. Public Health Service, Bethesda, Md.

JAY L. LUSH, Professor of Animal Breeding, Iowa State College, Ames, Iowa.

H. J. MULLER, Professor of Zoology, Indiana University, Bloomington, Indiana.

HUGH C. McPHEE, U. S. Department of Agriculture, Retired. Home address: 6514 40th Avenue, Hyattsville, Maryland.

HAROLD H. PLOUGH, Department of Biology, Amherst College, Amherst, Massachusetts.

MARCUS M. RHOADES, Professor of Botany, University of Indiana, Bloomington, Indiana.

W. RALPH SINGLETON, Director, Blandy Experimental Farm, Boyce, Virginia.

LAURENCE H. SNYDER, President of the University of Hawaii, Honolulu, Hawaii.

ADVISORY COMMITTEE

R. A. BRINK, Professor of Genetics, University of Wisconsin, Madison.

LEONARD BUTLER, Professor of Genetics, University of Toronto, Canada.

TH. DOBZHANSKY, Professor of Zoology, Columbia University, New York City.

C. M. GOETHE, Sacramento, California.

JOHN W. GOWEN, Professor of Genetics, Iowa State College, Ames.

C. N. HERNDON, Dir., Out-Patient Dept., Bowman Gray School of Medicine, Winston-Salem, N. C.

HARRISON R. HUNT, Professor of Zoology, Michigan State College, East Lansing.

D. F. JONES, Plant Breeder, Connecticut Agricultural Experiment Station, New Haven.

M. A. JULL, Professor Emeritus of Poultry Husbandry, University of Maryland, College Park.

F. J. KALLMANN, M.D., Professor of Psychiatry, Columbia University and Principal Research Scientist (Medical Genetics), New York State Psychiatric Institute, New York City.

C. E. KEELER, Professor of Zoology, Georgia State College for Women, Milledgeville.

L. W. LAW, Senior Scientist (Geneticist), National Cancer Institute, Bethesda, Md.

PHILIP LEVINE, Director, Biological Division, Ortho Research Foundation, Raritan, N. J.

C. C. LITTLE, Director Emeritus, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

C. P. OLIVER, Professor of Zoology, University of Texas, Austin.

L. F. RANDOLPH, Professor of Botany, Cornell University, Ithaca, N. Y.

PAUL B. SAWIN, Research Associate, Jackson Memorial Laboratory, Bar Harbor, Maine.

KARL SAX, Professor of Botany, Harvard University, Arnold Arboretum, Jamaica Plain, Mass.

EDMUND W. SINNOTT, Professor Emeritus of Botany, Yale University, New Haven, Conn.

RAMSAY SPILLMAN, M.D., New York City.

B. L. WARWICK, Professor of Animal Husbandry, Texas Agri. Expt. Station, College Station.

P. W. WHITING, Zoological Laboratory, University of Pennsylvania, Philadelphia.

G. W. WOOLLEY, Head, Division of Steroid Biology, Sloan-Kettering Institute, New York City.

CONWAY ZIRKLE, Professor of Botany, University of Pennsylvania, Philadelphia.

The JOURNAL OF HEREDITY is published bi-monthly by the AMERICAN GENETIC ASSOCIATION at 32nd Street and Elm Avenue, Baltimore 11, Md., and 1507 M St., N. W., Washington 5, D. C. Address all communications regarding editorial matters to 1507 M St., N. W., Washington, D. C. Communications regarding the business management of THE JOURNAL OF HEREDITY may be addressed to the publication office, 32nd Street and Elm Avenue, Baltimore, Md., or to 1507 M St., N. W., Washington, D. C. Entered as second-class matter February 24, 1923, at the postoffice at Baltimore, Maryland. Acceptance for mailing at the special rate of postage provided for in section 1103, Act of October 3, 1917, authorized December 29, 1918.

Contents copyrighted 1959 by the American Genetic Association. Unauthorized reproduction of material from THE JOURNAL not permitted. The title "Journal of Heredity" is registered as a trade-mark in the U. S. Patent Office.

$8.00 a Year. $2.00 a copy. Prices of back numbers on application.

The Journal of Heredity

Vol. L JULY-AUGUST, 1959 No. 4

CONTENTS

THE MITOTIC CHROMOSOMES OF PARTHENOGENETEIC AND NORMAL TURKEYS
By H. K. Poole 151

BLOOD-pH DIFFERENCES IN TWO INBRED STRAINS OF MICE
By H. G. Wolfe 155

MULTI-OVARY IN BARLEY
Om P. Kamra and R. A. Nilan 159

A MACROMUTATION IN YELLOW LUPINE
By J. R. Edwardson and M. K. Corbett 167

IRREGULAR MOSAICISM IN A HEN
By A. G. Cock 171

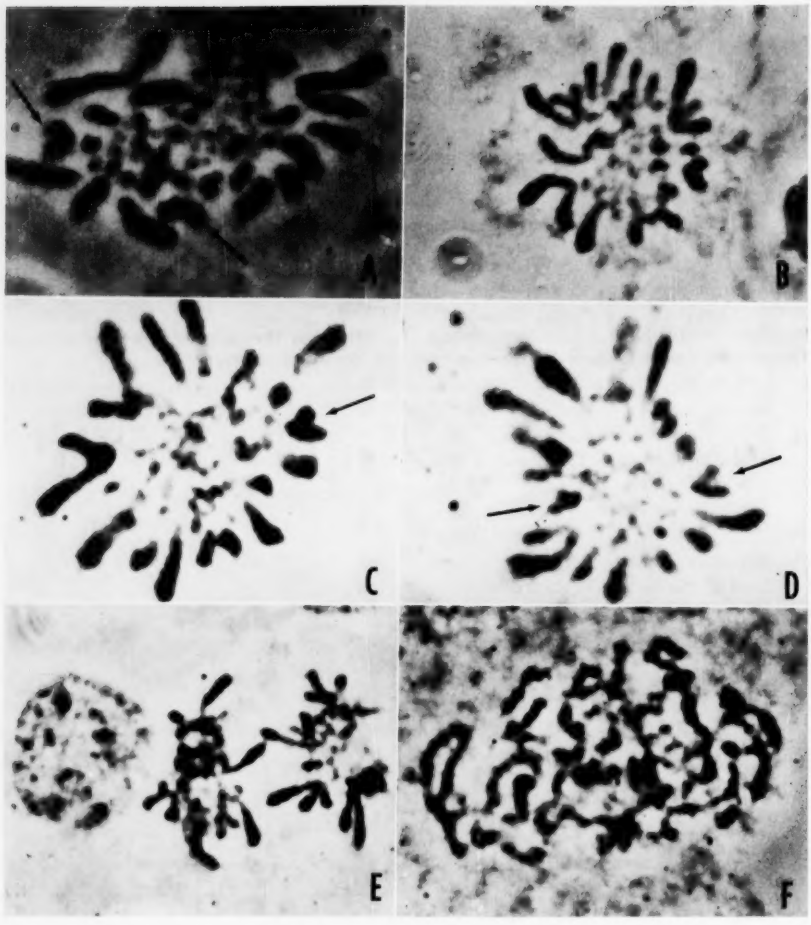
CYTOLOGICAL AND GENETIC CHANGES INDUCED BY VEGETABLE OILS IN TRITICUM
By M. S. Swaminathan and A. T. Natarajan 177

A MUTATION CAUSING CONGENITAL CLUBFOOT IN THE HOUSE MOUSE
By M. W. Robins 188

NOTES AND REVIEWS

'60 CENSUS TO LIST MALFORMED IN U.S. 154

A CASE OF TWIN EMBRYOS OF THE GUINEA FOWL
By Joseph C. Daniel, Jr. 176



TURKEY CHROMOSOMES

Figure 1

All photographs are phase contrast photomicrographs of squashed collar cells from pinfeathers of parthenogenetic and normal Beltsville Small White turkeys (magnification 1212×). Figure *A* is from parthenogenetic progeny of previously mated hen; *B*, *E* and *F* are from parthenogenetic progeny of virgin hens and; *C* and *D* are from normal progeny from normally mated hens. *A* and *B*—Metaphase configurations from parthenogenetic males with approximately 18 macrochromosomes. In *A* arrows indicate possible sex chromosomes. *C*—Metaphase from normal turkey female. Note microchromosomes appear poorly defined and sticky with interconnecting chromatin strands. Arrow indicates possible sex chromosome. *D*—Metaphase from normal turkey male. Note again the microchromosomes appear to be diffuse and sticky, with interconnecting strands of chromatin. Arrows indicate possible sex chromosomes. *E*—Telophase configuration from parthenogenetic male showing precocious loss of microscopic identity by the microchromosomes. Interphase nucleus shows many heterochromatic bodies. *F*—Early prophase from parthenogenetic male showing chromosomal condensation, heterochromatic bodies (possibly nucleoli) associated with the chromosomes, and early microchromosomes.

THE MITOTIC CHROMOSOMES OF PARTHENOGENETIC AND NORMAL TURKEYS

H. K. Poole*

WITH the discovery of parthenogenetic development in unfertilized Beltsville Small White turkey eggs[13,14], the question was raised as to whether the cells from such development would be haploid or diploid. Yao and Olsen[22] observed chromosome numbers ranging from 60 to 82 in cells of undifferentiated embryonic membranes of parthenogenetic origin. They concluded that these cells were diploid since their chromosome numbers were of the same order as those reported by Werner[21] (76 male and 77 female). More recently, Sato and Kosin[17] have reported the diploid number in normal turkeys as 18 macrochromosomes for the male and 17 for the female plus about 60 microchromosomes for both sexes. Their observations on cells of parthenogenetic origin lead them to state, ". . . counts on embryos and undifferentiated tissue from virgin eggs show nearly all nuclear plates of mitotic metaphase to be of diploid configuration." Also, Kosin *et al.*[2] have reported that metaphase plates from 15 day normal turkey embryos show a complement of 81 chromosomes, composed of 19 macrochromosomes and 62 microchromosomes. Their parallel observations on tissue of parthenogenetic origin show nearly all metaphase plates to have diploid chromosome sets.

The work described here was undertaken to 1) extend the observations of Yao and Olsen[22] to dividing cells in postembryonic parthenogentic turkeys and 2) to report any observable differences between the chromosome complements of parthenogenetic and normal turkeys. This study was completed before publication of the work by Kosin *et al.*[2] and Sato and Kosin[17] and constitutes independent confirmation of their findings.

Materials and Methods

Four parthenogenetic Beltsville Small White turkeys were used in these experiments. Two were hatched from eggs laid by virgin hens, and two were hatched from eggs laid by hens which were mated some eight months earlier, but not thereafter. (Seventy-two days is the longest period turkeys have been known to retain fertility following a normal mating[3].) All of these birds were males (as expected[15]), beyond twenty weeks of age, and were seen to strut characteristically. Three had yielded small amounts of semen containing motile spermatozoa[10].

Parallel observations were made on randomly selected normal Beltsville Small White turkeys whose tissues were handled in the same manner as those from the parthenogenetic individuals.

Following the technique of Sandnes[16], developing pinfeathers were plucked, pretreated in distilled water at room temperature for 15 minutes[4], and fixed in either of two fixatives recommended by Newcomer[6,7]. If the pinfeathers were not to be used immediately they were refrigerated at 0 degrees centigrade. Otherwise, following at least three hours in the fixative, the pinfeathers were placed in 45 percent acetic acid for 15 to 20 minutes. Next the ectodermal collar from the pinfeather base was removed and placed in Newcomer's staining mixture[6]. After one-half hour in the stain small bits of this tissue, containing actively dividing cells, were squashed in

*United States Department of Agriculture, Agriculture Research Service, Animal Husbandry Research Division, Beltsville, Maryland. The author wishes to thank Dr. M. W. Olsen for making the turkeys for this study available.

45 percent acetic acid and then studied and photographed by phase contrast microscopy.

Observations

No morphological or numerical differences were observed between the chromosome complements of cells from normal and parthenogenetic turkeys. The chromosome complements of all turkeys were found to be composed of two distinctly different types of chromosomes; 1) a group of large chromosome units, generally termed macrochromosomes, and 2) a group of much smaller units, generally termed microchromosomes (Figure 1*A*). These correspond directly to the two groups found to exist in chickens and which were termed "chromosomes" and "chromosomoids" respectively by Newcomer[8]. The macrochromosome group is composed of an average of 18 large units which are variably heterochromatic and which appear to divide with the regularity of behavior expected of true chromosomes. On the other hand, the microchromosome group is composed of from 10 or less to 50-60 small telocentric (or possibly acentric) units which may be individually discrete or may clump together to form larger aggregates. These microchromosomes are highly heterochromatic and in some instances (particularly prophase and metaphase configurations) appear to be attached to each other or to the macrochromosomes by thin strands of chromatin material (Figure 1*A-D*).

Since the mitotic stages in turkeys do not seem to vary greatly from Newcomer's description of mitosis in the chicken[8], they will be only briefly described here. In the interphase turkey cell the nucleus contains the many heterochromatic chromatin bodies, of various sizes, so typical of the avian nucleus (Figure 1*E*). These heterochromatic bodies, which may be true nucleoli, persist into the early prophase and appear to be associated with the condensation of the chromosomes (Figure 1*F*). As the prophase chromosomes condense the macrochromosomes exhibit a highly heterochromatic and heteropyknotic structure, and until late prophase are poorly defined from the reticular aggregation of the microchromosomes (Figure 1*F*).

Metaphase is the most common mitotic stage found on any given slide. By early metaphase the macrochromosomes have become arranged in a ring with the microchromosomes located in the center (Figure 1*A-D*). The macrochromosomes are highly heterochromatic in the early metaphase cell but as coiling and condensation is completed this property seems to be altered, so that at middle metaphase they appear to be of nearly uniform density (Figure 1*A*). At still later metaphase, when the individual chromatids become apparent, the macrochromosomes become heterochromatic once again and assume a banded type of morphology. The microchromosomes are rather consistently heterochromatic throughout the entire metaphase. Clumping of the microchromosomes and variability in their numbers is common (Figure 1*A-D*).

The anaphase stage was rarely found in this study, and thus apparently passes very rapidly. They are so rare, in fact, that of the few found none were satisfactory for photographic reproduction. In one isolated instance an anaphase was observed which suggested the formation of a heterochromatic chromatin bridge. This may have been an artifact due to the squash technique or it may have been a true chromatin bridge.

Telophase appears to be typical but such stages are also rarely found, though not nearly so rarely as anaphases. Precocious division of the microchromosomes is indicated by the fact that they begin to lose their individual identity much earlier at telophase than do the macrochromosomes (Figure 1*E*). Some instances of chromatin bridging may be seen in early telophase.

There is indication that the sex chromosome in turkeys is the sixth largest macrochromosome: paired in the male and unpaired in the female (Figure 1 *C* and *D*), although the validity of this observation needs confirmation.

Discussion

These observations on postembryonic tissue

and the results of previously cited authors in embryonic tissues[2, 17, 22] raise the question of how the diploid chromosome complement is established in parthenogenetic turkey tissues. Although Beatty[1] discussed the following cytological mechanisms by which diploid parthenogenesis may arise, he made no reference to the turkey. Diploid parthenogenesis may be the result of 1) suppression of meiosis I or reentry of the first polar body into the secondary oocyte followed by a reductional meiosis II; 2) suppression of meiosis II or reentry of a second polar body; or 3) nuclear but not cytoplasmic division of the mature haploid ovum with subsequent reconstitution of a diploid nucleus. Assuming the normal XX-XO sex chromosome relationship in birds and the lethality of a OO sex chromosome relationship (corresponding to the YY lethal in *Drosophila*[19]) one may conclude that these are the only cytological mechanisms which might explain diploidy in parthenogenetic turkeys.

Alternatives 1 and 2 cited above seem more likely since the prior observations of Olsen[11] in chickens and Olsen and Fraps[12] in turkeys were that following maturation of the ovum the polar bodies remain inside the vitelline membrane. However, confirmation of such mechanisms will come only after linkage data can be compiled using parthenogenetic males as sires, and after a thorough cytological study of the maturation of the turkey ovum.

The relationship of 18 macrochromosomes to ca. 60 microchromosomes is nearly the same as that reported by Kosin *et al.*[2], and Sato and Kosin[17]. Furthermore, Werner[21] found 12 large chromosomes, six short rod-shaped chromosomes, and 58 globular chromosomes; yet when one studies her illustrations it becomes apparent that these chromosome complements could just as logically have been considered to consist of 18 macrochromosomes and 58 microchromosomes—a close approximation of the results reported here and by previous workers[2, 17, 18, 21, 22].

The major numerical inconsistencies observed in this study appeared in connection with the microchromosomes. This observation is in accord with Werner's prior description of similar conditions. The tenuous chromatin strands which are frequently observed connecting microchromosomes and macrochromosomes suggest that they either contribute chromatin to be incorporated into the macrochromosomes or they are produced by extrusion of chromatin (probably intercalary heterochromatin) from the macrochromosomes. Either might cause apparent variation, both in the size of the individual chromosomes and in the chromosomes number. Newcomer[8] has argued for the former possibility in chickens but there is little information in turkeys to support these alternatives. Yet it would seem that the latter might be refuted easily since tre microchromosomes are present in the early prophase before condensation of the macrochromosomes has progressed to any extent.

Heteroploidy, expressed as aneuploidy, has been observed previously in mammals[20] as well as in turkeys[2, 17, 20, 21] and chickens[8, 9]. Walker and Boothroyd[20] have discussed such findings in mammals and on the basis of their own experiments in the mouse and man have concluded that use of the squash technique is largely responsible for these deviations toward hypoploidy. They have conceded, however, that minor deviations may not be due to an artifact produced by the squash but may be a biologically based, true, aneuploidy. It is the present author's opinion that this theory might explain the heteroploidy (tending toward hypoploidy) observed in this study. This opinion is supported by Werner's[21] observation of hypoploidy in cells of classically sectioned and stained turkey gonadal tissue. She reported this condition as due to loss of some of the globular chromosomes. Such observation can be explained as a result of the mitotic instability of telocentric chromosomes[6], and indicates that true hypoploidy does exist in turkeys though the extent is not presently known.

Summary

The chromosome complements of parthenogenetic and normal Beltsville Small White turkeys are described, and it is concluded that there is no difference between the chromosomes of normal and parthenogenetic turkeys. Therefore, the parthenogenetic turkey is considered diploid. In both normal and parthenogenetic turkeys the average number of macrochromosomes is 18, while the number of microchromosomes, being much more variable, range from 10 to 60. Varying degrees of heteroploidy (generally tending toward hypoploidy) were observed in both parthenogenetic and normal tissues. Both macrochromosomes and microchromosomes are heterochromatic throughout much of the mitotic cycle. Thin chromatin strands can frequently be seen between microchromosomes and between microchromosomes and macrochromosomes. Some observations indicate the possibility that the sixth largest element, a V-shaped chromosome, may be the sex chromosome and that it is paired in the male (both parthenogenetic and normal) and unpaired in the female. Heteroploidy, macro-microchromosomal relationship, and the possible cytological mechanisms capable of giving rise to diploid parthenogenesis in turkeys are briefly discussed.

Literature Cited

1. Beatty, R. A. Parthenogenesis and Polyploidy in Mammalian Development. Cambridge Monographs in Experimental Biology No. 7 Cambridge University Press. 1957.

2. Kosin, I. L., Iwane Sato, and Helen Nagra. Studies of parthenogenesis in the domestic turkey and chicken. *Eleventh World's Poultry Congress.* Mexico City, Mexico. Sept. 21-29, 1958.

3. Lorenz, F. W. Onset and duration of fertility in turkeys. *Poultry Sci.* **29**:20-26. 1950.

4. MAKINO, SAJIRO and ISAO NISHIMURA. Water pretreatment squash technic. *Stain Tech.* 27:1-7. 1952.
5. MARKS, G. E. Telocentric chromosomes. *Amer. Nat.* 91:223-232. 1957.
6. NEWCOMER, E. H. Handling avian chromosomes. *Stain Tech.* 27:205-208. 1952.
7. ————. A new cytological and histological fixing fluid. *Science* 118:161. 1953.
8. ————. The mitotic chromosomes of the domestic fowl. *Jour. Hered.* 48:227-234. 1957.
9. ———— and JOHN W. A. BRANT. Spermatogenesis in the domestic fowl. *Jour. Hered.* 45:79-87. 1954.
10. OLSEN, M. W. Unpublished data.
11. ————. Maturation, fertilization and early cleavage in the hen's egg. *Jour. Morph.* 70:513-532. 1942.
12. ———— and R. M. FRAPS. Maturation, fertilization and early cleavage of the egg of the domestic turkey. *Jour. Morph.* 74:297-309. 1944.
13. ———— and S. J. MARSDEN. Embryonic development in turkey eggs laid 60-224 days following removal of males. *Proc. Soc. Exptl. Biol. Med.* 82:638-641. 1953.
14. ———— and S. J. MARSDEN. Natural parthenogenesis in turkey eggs. *Science* 120: 545-546. 1954.
15. POOLE, H. K. and M. W. OLSEN. The sex of parthenogenetic turkey embryos. *Jour. Hered.* 48:217-218. 1957.
16. SANDNES, GUNNAR C. A new technique for the study of avian chromosomes. *Science* 119:508-509. 1954.
17. SATO, IWANE and I. L. KOSIN. Chromosome complements in parthenogenetic turkey embryos. (abstract) *Proc. Tenth Internat. Congress of Genetics.* 2:249-250. 1958.
18. SHIWAGO, P. I. Über den Chrosomenkomplex der Truthennen. *Z. f. Zellfarsch. u. Mikros. Anat.* 9:106-115. 1929.
19. STALKER, H. D. Parthenogenesis in Drosophila. *Genetics* 39:4-21. 1954.
20. WALKER, B. E. and E. R. BOOTHROYD. Chromosome numbers in mouse and man. *Genetics* 39:210-219. 1954.
21. WERNER, ORILLA S. The chromosomes of the domestic turkey. *Biol. Bull.* 61:157-164. 1931.
22. YAO, T. S. and M. W. OLSEN. Microscopic observations of parthenogenetic embryonic tissues. *Jour. Hered.* 46:133-134. 1955.

✦

'60 CENSUS TO LIST MALFORMED IN U. S.

A national tabulation of malformations at birth will be started in 1960 by the Office of Vital Statistics.

Joseph Schachter, chief of the Natality Section, confirmed today that the move resulted largely from the wide interest in the hereditary effect of radioactivity.

Some months ago a geneticist and a geologist from the Atomic Energy Commission sought Mr. Schachter's aid for a study of geographic differences in congenital malformations as possibly correlated with surface radiation.

Mr. Schachter said today that this specific inquiry, together with the popular and scientific interest shown in this and other phases of genetics, had resulted in the opening of the new area in national vital statistics reporting.

He said that the continuing tabulation will start in 1960 with a simple tally of the congenital malformations reported by the more than forty states that carry a question on that subject on the birth certificate.

He said, however, that the aim would be to refine and broaden the reporting until the statistics could become of great value in answering many of the questions puzzling geneticists, including the actual effects of radiation on future generations.

Already many states ask for a description of the congenital malformation. Such descriptions could lead to a classification of types of malformation. Some are hereditary; some are accidents in the reproductive process, and some may be radiation damage resulting in mutations.

Congenital amputations are one field in which the new reporting would prove valuable, entirely apart from atomic energy considerations. Sometimes children are born lacking part of a leg or arm, which is not believed to be hereditary.

Sometimes, however, the congenital amputation is in the form of a "lobster claw" type of hand or vestigial foot, both conditions regarded as hereditary. Nobody now knows the prevalence of such cases in the population, or whether they are increasing or decreasing.

Other vistas might be opened up by

(*Continued on page* 165)

BLOOD-pH DIFFERENCES IN TWO INBRED STRAINS OF MICE

H. G. WOLFE*

THE objectives of this investigation were to compare pH of arterial and venous blood, and to re-examine the nature of strain differences in two inbred strains of the common laboratory mouse, *Mus musculus*. The strains were derived initially by selection for high and low blood-pH[11]. One point in question was whether the two strains still exhibited pH differences obtained by Weir and Clark at the termination of their selection. Secondly the information gained might give the investigator a better index for the selection of desired parents in a concurrent selection experiment. As reported by Weir and Clark[11], the mean difference between high and low blood-pH lines was .050 pH units after three generations of selection. Most progress was accomplished in the first generation of selection (mean difference = .046), indicating to the investigators a relatively simple genetic model. No significant sex difference in blood-pH was found nor did age have any appreciable effect[10,11].

Hawkins[3] reported mean blood-pH values for guinea pigs, rabbits and rats obtained by the colorimetric method and converted to body temperatures. Arterio-venous difference for the guinea pig was .115; for the rabbit it was .071. No comparison between arterial and venous blood is presented for the rat, since blood samples were obtained by decapitation and readings obtained for mixed blood.

The *Handbook of Biological Data*[9] lists pH values of several animals, at body temperature, including the guinea pig, rabbit, rat and hamster. There are no values for the house mouse, however, and arterial and venous means are one or the other lacking in most animals, excluding man, so that a comparison cannot be made. Arterio-venous blood-pH difference in man is generally recognized as being about .02 to .03 units[6].

Weir[10] found a difference in pH of .115 between arterial and venous blood using four different inbred strains of mice, including some F_1 progeny of strain crosses. Comparisons were made between sib pairs.

Although blood-pH is well buffered by hemoglobin, plasma proteins, and carbonate and phosphate systems, there is still considerable variability within a rather narrow range compatible with life. In addition to buffers in the blood there are several compensatory mechanisms in the body, such as the excretory and respiratory systems[1]. Metabolic disorders, ingestion of acid or alkaline foods and severe exercise[4] can all serve to cause a temporary change in blood-pH which, in these exigencies, can only be partially buffered back to normal. That there is much individual variation in the acid-base balance of the blood was confirmed by Shock and Hastings[8] in their studies of human blood. They also found a slightly higher pH in women than in men.

Blood-pH *in vitro* varies inversely with temperature. Conversion factors for each degree Centigrade change in human blood have been variously estimated as being .011 to .020[2]. Rosenthal[5] found a coefficient of .0147 pH units for each degree Centigrade change in his investigations of the effect of temperature on whole human blood *in vitro*. Mean temperature coefficient of dogs was .0107 compared to .0138 for rabbits for plasma or serum samples[5].

Temperature effects on mouse blood

*Contribution from the Hall Laboratory of Mammalian Genetics, Department of Zoology, the University of Kansas, Lawrence. This investigation was supported by research grant CY 3655 from the National Institutes of Health, United States Public Health Service. The author gratefully acknowledges the advice and encouragement of Dr. J. A. Weir throughout the experiment and the helpful criticisms offered by Dr. D. W. Bailey in the preparation of the manuscript.

appear to be complicated by an effect on the mouse *in toto*, as well as a direct effect on the blood itself[10]. Mice kept at a low temperature but tested at a higher temperature registered a lower pH than the controls. This might have been due to an increased activity on the part of the mice[10].

Materials and Methods

The two strains of mice used in the experiment are designated pHH (high blood-pH) and pHL (low blood-pH). They have been maintained since the fourth selected generation up to the present 17-24 generations almost exclusively by full-sib matings.

Sib pair mice were sampled in units of two, i.e., one reduced and one oxygenated, and alternated by strain. The mice were retested after an interval of 14-43 days; in most cases the same type of blood was obtained as in the first test. All data reported are from male mice except for the inclusion of six females.

Mice were tested between the ages of two and 7½ months. No mice were tested before two months of age since there is a higher mortality among the younger and smaller mice.

A research type Cambridge pH Meter equipped with a McInnes Belcher microglass electrode of 0.4 cc capacity and with an overall accuracy of ±.02 units was used in the blood-pH determinations. The machine was standardized on a potassium and sodium phosphate buffer solution of 7.38 and checked with two other standard buffer solutions of 4.00 and 4.64. This procedure was completed at the beginning of each series of determinations and checked periodically during the series. If a change of 1-2°C in room temperature occurred, the machine was completely restandardized at that temperature. Room temperature range over the duration of the experiment was 24-32°C and usually varied no more than 3°C on any one day of test.

Method of blood extraction was that employed by Weir[10]. Samples were obtained by cardiac puncture, sufficient skill having been attained to permit selection of the right or left ventricle for venous or arterial blood, respectively, with about 80 percent accuracy. Blood samples of 0.5 cc were collected from non-anaesthetized mice using a 0.5 cc tuberculin syringe. The whole blood in the syringe was transferred directly into the glass electrode with minimal loss of CO_2 content. No anticoagulants were added to the blood but before the taking of each sample, the syringe and needle were rinsed in a three percent solution of potassium oxalate.

Three items of major concern in a blood-pH determination are temperature effects, loss of carbon dioxide from the blood sample, and glycolytic reactions[2]. Using the blood sampling procedure outlined, there was negligible loss of carbon dioxide and little time for glycolytic reactions[1]. Effects of temperature are held constant by use of regression in the analyses.

The problem of pH measurement involved both the measurement *per se* plus the technique of handling the blood sample. Maximum accuracy is obtained when the time interval between drawing the sample and the reading of the pH is minimal[6]. The usual interval of time required to fill the syringe and obtain a reading was 63±10 seconds.

In a recent experiment, Severinghaus *et al.*[7] found a "suspension effect" error of whole blood when using the McInnes

TABLE I. Mean blood-pH by strain and type blood

Strain	Type blood	N	Mean blood-pH	Mean temp. (°C)	Mean age (days)
pHH	Arterial	50	7.480±.0062	29.3	128.4
	Venous	42	7.428±.0065	29.8	123.7
pHL	Arterial	37	7.475±.0076	29.2	121.2
	Venous	40	7.381±.0084	29.5	125.6

Belcher glass electrode. The correction factor is to add .01 to each pH reading. While the correction is recognized, it is not used in the data reported herein.

Experimental Results

Means and standard errors of the blood-pH for high (pHH) and low (pHL) line mice are given in Table I. Mean temperature of the blood approximated 29.5°C with a range of 24-32°C, and mean age was about 125 days with a range of 69-219 days for strains and types of blood combined.

To remove effects of temperature and age and provide a more suitable basis for comparing strains and arterio-venous means, the data were subjected to a regression analysis. Partial regression coefficients are given in Table II. There is a definite temperature effect and partial regression coefficients are negative in each case. This confirms observations of other investigators[2,5] that pH of blood *in vitro* has an inverse relationship to temperature. A linear relationship is also indicated. Regression of pH on age is not significant. Two coefficients are negative and two are positive; difference in signs can be explained as due to sampling error.

Since the effect of age is not statistically significant, it is disregarded in the pooled data. Strains and arterial and venous blood are combined so as to give an estimate of the temperature regression coefficient which is most representative of the population. From this analysis, the temperature regression coefficient is estimated to be —.007.

In Table III, data are arranged by sib pairs, one of each pair yielding arterial blood and the other venous blood. Thus one would be comparing mice of like genotype less the effects of sub-lines and more critical analyses could be made. Difference between pHH arterial and pHH venous means is .053 compared to .091 for pHL. Both values are highly significant. The data indicate a real difference in the arterio-venous blood-pH picture between pHH and pHL strains. The pHH arterio-venous difference differs significantly from the pHL arterio-venous difference. It can be seen that nearly all of this inter-strain difference (.038) comes from a difference in the venous blood between the two lines. When this same population of mice was retested, these differences, but not as extreme, were again obtained and in the same direction in each case.

A difference of .050 pH units between means of pHH and pHL strains was reached after three generations of selection by Weir and Clark[11], using venous blood. This compares favorably to the present difference between pHH venous and pHL venous blood of .043.

Discussion

The inverse linear relationship of temperature and blood-pH *in vitro* follows the accepted pattern and needs no additional comment. The regression coefficient of —.007 is considerably lower than the mean temperature coefficient of Rosenthal[5] for humans (.0147) but not so different from that of dogs (.0107).

Weir[10] found no appreciable age effect on blood-pH. Within the age range of mice used in this experiment, there was likewise no age effect demonstrated. It would be expected that age affects blood-pH in a manner correlated with changes in body metabolism. It has been shown in humans by numerous investigators[9] that pH varies inversely with age as does metabolic rate.

The inter-strain difference in venous blood-pH of .043 (Table III) compares favorably with the .050 selection difference obtained by Weir and Clark[11] and indicates that there was little regression after selection ceased. Arteno-venous difference for the pHH strain is .053 compared to .091 for pHL (Table III). These values approximate that of the rabbit (.071)[3] and are somewhat less than the mean arterio-

TABLE III. Mean pH* of arterial and venous blood from sib pairs of pHH and pHL mice

Strain	No. of sib pairs	Arterial	Venous	Difference
pHH	32	7.478	7.425	.053**
pHL	27	7.473	7.382	.091**
Difference		.005	.043**	/.038/**

*All pH values have been corrected to 29.5°C by the conversion factor .007.

**Significant at the .001 level of probability.

TABLE II. Regression of blood-pH on temperature and age

Strain	Type blood	N*	Partial regression coefficient	
			pH on temp. (°C)	pH on age (days)
pHH	Arterial	91	—.007**	.0001
	Venous	84	—.004	.0001
pHL	Arterial	62	—.003	—.0001
	Venous	81	—.007**	—.0002

*Includes retested mice

**Significant at the .01 level of probability

venous difference of .115 reported by Weir[10] for four inbred strains of mice and their F_1 progeny. Selection using venous blood is postulated as the reason for the greater spread between arterial and venous blood -pH in the pHL line.

The question naturally arises whether one can change venous blood-pH by selection without selecting arterial blood-pH as a closely correlated change. The greater arterio-venous difference in the low line is contingent upon the idea that one can. One would expect a high degree of correlation between arterial and venous blood-pH and would suppose the difference to be a fairly constant factor; yet it seems likely that one could change the oxygen-carbon dioxide carrying potential and produce a difference, since CO_2 gives to venous blood its less alkaline qualities. Pursuing this argument further, there must be factors whose main effects are for high venous blood-pH, those whose main effects are for low venous blood-pH, and factors common to both. The same thing applies to arterial blood. Added support for this idea comes from the author's selection experiment currently in progress[12]. High and low blood-pH lines are again being selected from the same foundation stock except that arterial blood is being used. Data through five generations of selection show a greater arterio-venous difference in the high line.

There is one additional fact which should be mentioned. Following transfer of the strains from the University of Saskatchewan to the University of Kansas, the pHH line mice were perpetuated by a single pair mating and the pHL line mice by three males and four females in the S-6 generation[11]. The reduction in numbers of sublines with subsequent loss of genes for high arterial blood-pH in the pHH line might account for some of the difference.

Conclusions and Summary

Arterial and venous blood-pH of two highly inbred mouse strains derived initially by selection for high blood-pH (pHH) and low blood-pH (pHL) are compared. Mean arterio-venous difference of pHH is .053 pH units compared to .091 pH units for pHL, giving a significant inter-strain difference of .038. This stems from a significantly greater difference between venous blood-pH of the two strains over that of arterial blood-pH (.043 for venous cf. .005 for arterial). Selection having been based on venous blood is postulated to be the reason for the differences between strains. Two-way selection for high and low venous blood-pH had the effect of reducing the arterio-venous difference in the high line and magnifying the arterio-venous difference in the low line. Results from a selection experiment currently in progress favor this hypothesis. High and low blood-pH lines derived from the same foundation stock but using arterial blood show a significantly greater arterio-venous difference in the high line through five generations of selection.

The inverse linear relationship between temperature and blood-pH *in vitro* is confirmed. The coefficient for each degree Centigrade change is estimated by regression analysis to be —.007.

There is no effect of age on pH as shown by regression analysis under conditions of the experiment.

Literature Cited

1. Davenport, H. W. The ABC of Acid-Base Chemistry. Univ. of Chicago Press. 1958.

2. Harris, I., E. L. Rubin and W. J. Shutt. Modifications in the use of the glass electrode for the determination of the pH of venous blood. *Jour. Physiol.* 81:147-152. 1934.

3. Hawkins, J. A. The acid-base equilibrium of the blood of normal guinea pigs, rabbits and rats. *Jour. Biol. Chem.* 61:147-155. 1924.

4. Heusner, W. W. Jr. and E. M. Bernauer. Relationship between level of physical condition and pH of antecubital venous blood. *Jour. Appl. Physiol.* 9:171-175. 1956.

5. Rosenthal, T. B. The effect of temperature on the pH of blood and plasma in vitro. *Jour. Biol. Chem.* 173:25-30. 1948.

6. Sendroy, J. Jr. Problems in measurement of pH of blood and other biological fluids. American Society for Testing Materials Symposium on pH Measurement Tech. Pub. No. 190, Phila. 1957.

7. Severinghaus, J. W., M. Stupfel and A. F. Bradley. Accuracy of blood pH and pCO_2 determinations. *Jour. Appl. Physiol.* 9: 189-196. 1956.

8. Shock, N. W. and A. B. Hastings. Studies in the acid-base balance of the blood. III. Variations in the acid-base balance of the blood in normal individuals. *Jour. Biol. Chem.* 104:585-600. 1934.

9. Spector, W. S. ed. Handbook of Biological Data. W. B. Saunders Co., Phila. & London. 1956.

10. Weir, J. A. Blood-pH as a factor in genetic resistance in mouse typhoid. *Jour. Infect. Dis.* 84:252-274. 1949.

11. ———— and R. D. Clark. Production of high and low blood-pH lines of mice by selection with inbreeding. *Jour. Hered.* 46: 125-132. 1955.

12. Wolfe, H. G. Unpublished data. 1958.

MULTI-OVARY IN BARLEY

Floral Anatomy and Embryo-sac Development

OM P. KAMRA AND R. A. NILAN*

PISTILLODY, or conversion of stamens into pistils, has been reported in many species of plants for nearly a century (c.f. Masters[12]; Worsdell[16]). In most examples the transformation is under the control of environment and is not inherited[1,5,9,10,11]. A few cases, however, show it to be genetically controlled[3] and inherited as a single recessive[4,15] or as a single dominant[15].

In recent years there has been a revival of interest in studies of X-ray induced mutants in which reversal of sex organs has occurred. From detailed anatomical studies of these mutants greater understanding of gene action and differentiation of sex organs in higher plants may be obtained. However, anatomical changes associated with pistillody have not been reported. The present paper describes the floral anatomy and embryo-sac development in a pistilloid mutant of barley called multi-ovary[7,8,13].

The transformation of stamens into more or less complete carpels has been reported in common wheat[1,11] and barley[5,9]. Goodspeed[4] observed a partial transformation of stamens into pistils in the second generation of X-ray treated *Nicotiana tabacum*. This anomaly was due to a single recessive gene and was given a gene symbol of "stigmoid" (*sg*).

Pistillody has been reported frequently in intergeneric hybrids involving *Triticum vulgare* as one of the parents. Kihara[10] found this phenomenon in *Aegilops caudata* × *T. vulgare* hybrids and believed it to be governed by day length. The transformation was incomplete and no more than one seed per floret was produced. Dr. Horst Gaul (personal communication) has observed similar transformation in *T. vulgare* × *Agropyron elongatum* hybrids. Recently Tsitsin and Lubimova[14] have described pistillody in florets of hybrid plants from crosses between perennial wheats *Triticum agropyrotriticum perenne* (Cicin) and either *A. glaucum* or *A. elongatum*. Transformations were also observed in *A. glaucum* × *A. elongatum* hybrids. However, when *T. vulgare* was one of the parents no such anomalies were observed. Florets with three normal anthers and up to three pistils were also observed. Hybridization between distinctly related species was suggested as the stimulus for the enhancement of the mutation process giving rise to these abnormalities.

Materials and Methods

The multi-ovary condition was induced in the variety Trebi (*Hordeum vulgare*) by atomic bomb irradiation at Bikini in 1946 and is characterized by the absence of stamens and an increased number of pistils. This sex-altered mutant behaves as a simple recessive and has a designated gene symbol of *mo*[7,8,13].

Families segregating for multi-ovary were grown in the field in the summer of 1957. Spikes of normal and mutant barley containing stages ranging from early microsporogenesis to embryo-sac development were collected and fixed in a solution of formalin, acetic acid and alcohol (FAA). The material was embedded in paraffin. Both longitudinal and transverse serial sections were cut 10 micron in thickness and were stained

*Institute of Genetics, University of Lund, Sweden, Genetics Department, Forest Research Institute of Sweden, Stockholm; and Department of Agronomy, Washington State University, Pullman, Washington. Scientific Paper No. 1791. Washington Agricultural Experiment Stations, Pullman. Projects 1002 and 1068 supported in part by U. S. Atomic Energy Commission Contract No. AT (45-1)-353 and funds provided for Medical and Biological Research by the State of Washington, Initiative Measure 171. The authors wish to express their appreciation to Professor N. Higinbothan and Drs. T. S. Bakshi and H. C. Govindu for their suggestions and encouragements.

PHOTOMICROGRAPH OF PARTLY TRANSFORMED ANTHER

Figure 2

A stamen transformed into a half-anther, half-ovary structure.

with a quadruple staining technique[6].

Results

Pistillody

The conversion to the multi-ovary condition occurs very early in the development of recessive homozygous spikes. In fact, the conversion had already occurred even in florets in which partially transformed stamens were initiating microsporogenesis. A detailed morphological and genetical description of this mutant has already been published[13].

Normal barley has one pistil per spikelet. However, in *mo* plants each of the sporogenous units of a stamen can give rise to a pistil, resulting in more than four pistils per spikelet. Large converted pistils appeared to have several smaller ones growing or attached to them, making it difficult to ascertain the exact number of pistils.

LONGITUDINAL SECTION OF MEDIAN SPIKELET OF THE MUTANT

Figure 3

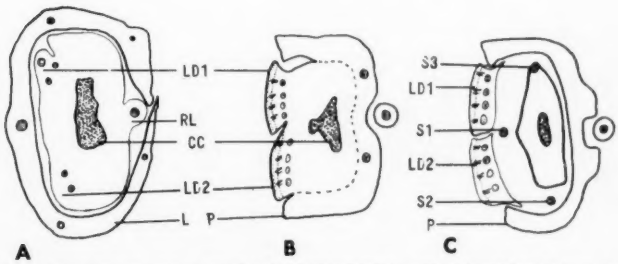
Normal ovary in the center with with one stamen completely transformed into an ovary and the other into a three-fourth ovary, one-fourth anther structure. The third stamen is not shown in the photograph.

The various types of transformations observed ranged from partial to complete conversion of stamens into pistils. Examples of these are pictured in Figures 2, 3 and 8. Figure 2 shows a stamen transformed into a half-anther, half-ovary structure. In Figure 3, the longitudinal section of the median spikelet of the mutant shows the true ovary in the center with one stamen completely transformed into an ovary and the other into a structure which is three-fourths ovary, one-fourth anther. The third stamen is not visible in the picture.

Floral anatomy of normal barley

The morphological development of various structures of two and six rowed barley spikes has been described by Bonnett[2]. However, it was necessary to study the floral anatomy and the embryo-sac development of the normal barley in order to interpret correctly the changes in the mutant.

The beginning of development and differentiation of the various structures of the spikelet is similar in both normal and multi-ovary barley. The main floral axis, the rachis, contains 15 to 20 vascu-



DEVELOPMENT OF MEDIAN SPIKELETS OF NORMAL BARLEY

Figure 4

CC—central cylinder, L—lemma, LD, LD1 and LD2—lodicules (1 and 2 indicate the order of differentiation), P—palea, RL-rachilla, S1,2,3—stamen. 1, 2 and 3 indicate the order of differentiation. See text for details.

lar bundles. Six or seven of these divide and give rise to the vascular supply of the spikelets whereas other vascular bundles fan out to the continuous rachis.

Vascular bundles of the spikelets give rise to two sets of vascular traces radially. These form the vascular supply of the two lateral spikelets. The two lateral spikelets develop in much the same way as the median spikelet. However, two differences exist. The median spikelet starts developing at a lower level so that traces from it supply the vascular needs of the two lateral spikelets and it is more developed than either of the two lateral spikelets.

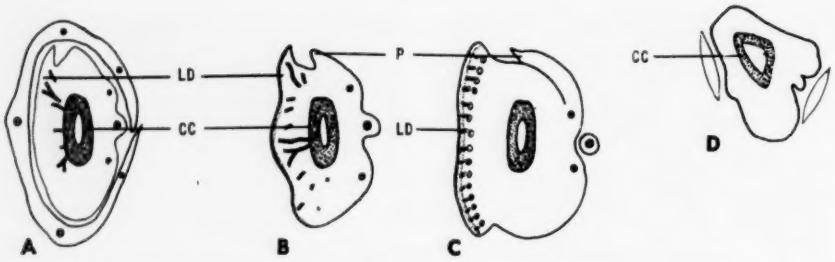
The order of differentiation and development of the floral structure of the three spikelets is: First, the floral structures of the median spikelet appear; second, the floral structures of the lateral spikelet I develop (on one side of the median spikelet); finally, the structures of the lateral spikelet II appear (on the other side of the median spikelet). Differentiation of the two glumes as well as the floral structures (lemma, palea and the lodicules) within all three spikelets starts at one end of the structure and proceeds to the other (The development of various structures as diagrammed in Figures 4 and 5 *A-C* starts at the top and proceeds to the bottom).

Development of median spikelets of normal barley

Vascular bundles of the central cylinder give rise to three abaxial traces on one side and two to the other. These are the traces for the first and second outer glumes, respectively. These glumes differ from the ordinary glumes of the grasses in that they do not face each other but lie against the lemma of the median spikelet (Figure 8*A*). After the differentiation of glumes the main vascular supply of the median spikelet gives off four abaxial traces, two on one side and two on the other. This is immediately followed by another abaxial median trace. These five traces form the vascular supply of the lemma (Figure 4*A*). At this level one adaxial median trace originates, followed by two more (one on each side); the first forms the vascular supply for the rachilla and the last two for the palea (Figure 4 *A* and *B*).

Before the rachilla becomes distinct from the mass of tissue of the median spikelet, three abaxial traces originate to one side and two to the other. These five traces constitute the vascular supply of the lodicules (Figure 4*A*). In Figure 4*B* the rachilla has separated, the palea has started to separate (note the differentiation starting on one side and proceeding to the other) and the central cylinder has produced a median abaxial trace for the first stamen.

The order of differentiation of vascular bundles for the three stamens can be seen in Figure 4 *B* and *C*. The residual vascular tissue constitutes the vascular supply for the pistil (Figure 6*A*). It



DEVELOPMENT OF MEDIAN SPIKELETS OF MUTANT BARLEY

Figure 5

A-C—Various stages in the development of floral structures of the median spikelet of the mutant. Compare with Figure 4. *D* shows the central cylinder of lateral spike 1 of the mutant. See Figure 7 and text for details.

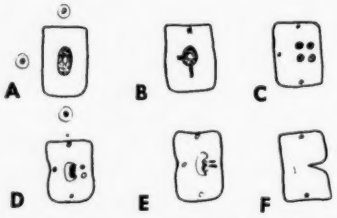
gives rise to three dorsal traces (Figure 6*B*). The remainder supplies the ovule (Figure 6*D* and *E*). The various stages of development of the ovary and the ovule are diagrammed in Figure 6. The ovary has one basal ovule and two short styles and stigmata. The number of visible traces confirm the tricarpellate origin of the barley ovary.

Anatomical differences between spikelets of normal and multi-ovary barley

The beginning of anatomical development and differentiation is similar in both normal and mutant barley spikelets. Minor differences occur in the shape of the central cylinder (Figure 4 and 5 *A-C*). In the normal, five abaxial traces arise from the central cylinder, three on one side and two on the other (Figure 4 *A*.

In the mutant, many traces all along the abaxial surface of the central cylinder arise (Figure 5 *A-C*). These constitute the vascular supply of the lodicule. Moreover, the lodicule in the median spikelet (Figures 5*C* and 8*B*) is always single and undivided whereas lodicules of the two lateral spikelets of the mutant (Figure 5*D*) as well as the spikelets of normal plants are two in number.

In Figures 5*D* and 7 the events that lead to the formation of the vascular supply for a structure which is one-fourth ovary, three-fourths stamen are reconstructed. The central cylinder



DEVELOPMENT OF MEDIAN SPIKELETS

Figure 6

The various stages of the development of the ovary and ovule are diagramed above. See text for details.

which would normally give a single trace for the filament divides and gives traces for both the filaments and the ovule.

Other anatomical differences between normal and mutant spikelets are:

(a) The rachis in the mutant is thicker than in normal barley.

(b) The lemma and palea of the mutant are thinner and have less vascular tissue than the normal (Figure 8).

(c) As a whole, the mutant seems to contain more vascular tissue than the normal.

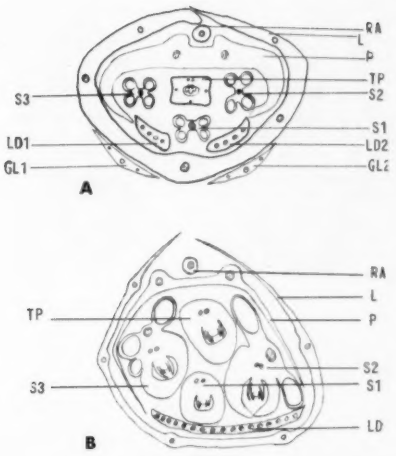
Embryo-sac development in mutant and normal florets

Various stages in the development of embryo-sac were observed in florets of

DEVELOPMENT OF MEDIAN SPIKELETS

Figure 7

The events that would lead to the formation of the vascular supply for one-fourth ovary, three-fourths anther structure are shown above.



COMPOSITE DIAGRAM OF MEDIAN SPIKELETS OF NORMAL AND MUTANT BARLEY

Figure 8

GL1 and GL2—glumes (1 and 2 indicate the order of differentiation), L—lemma, LD, LD1 and LD2—lodicules, P—palea, RL—rachilla, S1, 2, 3—stamen (In the mutant. *B*. the stamens have changed partly or completely), TP—true pistil.

both normal and mutant barley. The mature embryo-sac of both is of the polygonum type. However, it contains six antipodals (Figure 9*C*). A frequent abnormality observed in the mutant, following the second meiotic division, is the development of a T-shaped tetrad (Figure 9*A*). In normal barley only a linear tetrad of spores could be observed (Figure 9*B*). Two T-shaped configurations were found in 12 mutant tetrads studied whereas none occurred in 10 tetrads from normal plants.

In normal barley ovules are always enclosed. However, the ovules in ovaries from completely and partially transformed stamens are enclosed, semi-naked, or naked (Figure 8*B*).

Discussion

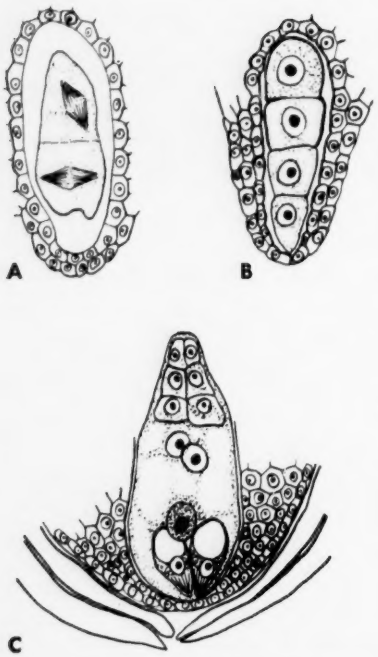
Previous reports on pistillody[1,3,4,5,9,10,11,12,-14,16] have shown it to be either incomplete or, if complete, the extra carpels are non-functional. In the present example the conversion of stamens into pistils is partial to complete. Since in this mutant more than one seed per spikelet can be obtained upon artificial pollination[13], it is necessary to assume that at least the completely transformed stamens produce viable seeds.

It is impossible to determine definitely if the pollen produced in partly transformed stamens is functional. However, up to 3.5 percent of 548 bagged florets set seeds under field conditions[13]. The majority of pollen undoubtedly came from non-transformed and normal stamens, although, some could have come from partly transformed stamens.

Increased vascular tissue of the lodicule provides greater possibility for seed set through cross pollination in the male sterile mutant plants. The modified lodicules open the florets early when the stigmata are receptive and keep them open for longer than normal periods. It is interesting to note that Moh and Nilan[13] found 13.8 percent of the unbagged florets and 3.5 percent of the bagged florets with seeds. This difference was attributed to open pollination. Thus the modified lodicules may be considered an adaptive change in the mutant plants and may have evolutionary significance.

In barley, the lodicules seem to be quite susceptible to change following irradiation. The senior author is at present studying five different single recessive barley mutants, produced by Dr. D. von Wettstein and coworkers in Sweden, in which lodicules are transformed into stamens.

The order of development and differentiation of floral structures of normal and mutant barley can be listed as lemma, palea, lodicules, stamens and pistil. This confirms the earlier



MEGASPOROGENESIS AND EMBRYO-SAC OF MULTI-OVARY BARLEY

Figure 9

T-shaped tetrad (*A*) as compared to linear tetrad in normal barley, (*B*). See text for details.

work of Bonnett[2] on morphological development of various structures of barley spikes.

The gene, *mo*, has produced a variety of effects, including semi-naked or naked ovules. The number and kinds of effects may have implications in macro-evolution, survival and adaptation, and plant classification.

The action of *mo* is not complete, particularly with regard to the amount of transformation of stamens, the modification of lodicules, the vascular tissue, and the nakedness of ovules. Thus this gene appears to have low expressivity. Greater variability of the expression in the morphology of the mutant was observed in a winter planting in the greenhouse. Therefore, it is suspected that microclimate, existing at a critical time of the conversion of stamens into pistils, may partly account for the low expressivity. For this reason, anatomical studies of this mutant under different environments may be helpful in understanding the action of this gene and, in turn, lead to a greater knowledge of sex differentiation in barley and other higher plants.

The results of this study point to the delicate balance in the differentiation of sex organs. Each of the four sporogenous units of an anther can give rise to an ovary, thus indicating the individuality of each anther sac.

The problem of differentiation of sex organs is an involved one in which the distinction of two sexes occurs very early in the development of spike primordia. The critical time at which this occurs, and its modifying factors still remain unknown. However, further controlled studies of this mutant should be helpful in an understanding of this important problem.

Summary

Floral anatomy, embryo-sac development, and other abnormalities in normal and multi-ovary (*mo*) barley were studied. The change to the multi-ovary condition occurs very early in the development of the spikelet. Each of the anther sacs can develop into an ovary, giving more than one seed per spikelet upon artificial pollination. The lodicules of the median spikelet of the mutant remain undivided, whereas those of the lateral spikelets are divided. A pair of lodicules occur in each normal spikelet. Possible significance of this lodicular change in the adaptation and evolution of multi-ovary barley is discussed.

As a whole, the mutant appears to possess more vascular tissue than the normal. In the normal and mutant barley the embryo-sac is of the polygonum type with three to six antipodals. Frequent abnormalities in the mutant are the T-shaped tetrads and naked or semi-naked ovules. The gene *mo* appears to have low expressivity. The value of this mutant for further studies on sex differentiation is discussed.

Literature Cited

1. Anthony, S. A. An anomaly of wheat anthers. *Jour. Hered.* 9:166-168. 1918.

2. Bonnett, O. T. The development of the barley spike. *Jour. Agric. Res.* 51:451-457. 1935.

3. De Vries, Hugo. Species and Varieties—Their Origin by Mutations. The Open Court Publishing Co., Chicago. pp. 847. 1912.

4. Goodspeed, T. H. Inheritance in *Nicotiana tabacum*. IX. Mutations following treatment with X-rays and radium. *Univ. Calif. Publ. Bot.* 11:285-298. 1940.

5. Gregory, F. G. and O. N. Purvis. Abnormal flower development in barley involving sex reversal. *Nature* 160:221-222. 1947.

6. Johansen, D. A. Plant Microtechnique. McGraw-Hill Book Co., New York. pp. 87-89. 1940.

7. Kamra, Om. P. Further studies on multi-ovary. *Barley Newsletter* 1:15-16. 1958.

8. ——— and R. A. Nilan. Anatomy of the multi-ovary mutant in barley. *Proc. X Int. Cong. Genet.* 2:139-140. 1958.

9. KARTAMYSEV, V. G. Changes in the reproductive organs of barley. *Agrobiolgija* (*Agrobiology*) 1:100-103. 1953. (Russian) *Pl. Br. Abst.* 23:2680.

10. KIHARA, HITOSHI. Substitution of nucleus and its effects on genome manifestations. *Cytologia* 16:177-193. 1951.

11. LEIGHTY, C. E. and W. J. SANDO. Pistillody in wheat flowers. *Jour. Hered.* 15:263-268. 1924.

12. MASTERS, MAXWELL T. Vegetable Teratology. Roberts Hardwicke 192, Picadilly, London 534 pp. 1869.

13. MOH, C. C. and R. A. NILAN. Multi-ovary in barley. A mutant induced by atomic-bomb irradiation. *Jour. Hered.* 44:183-184. 1953.

14. TSITSIN, N. V. and V. F. LUBIMOVA. New species and forms of cereals derived from hybridization between wheat and couch grass. *Amer. Nat.* 93:181-191. 1959.

15. WHITE, ORLAND E. Studies on teratological phenomenon in their relation to evolution and the problems of heredity. I. A study of certain floral abnormalities in *Nicotiana* and their bearing on theories of dominance. *Amer. Jour. Bot.* 1:23-36. 1914.

16. WORSDELL, W. C. Principles of Plant Teratology. Vol. 2. Dulau and Co., Ltd., London. pp. 182-193. 1916.

'60 Census to List Malformed in U. S.

(*Continued from page* 154)

the medical reporting even now being recorded on birth certificates. It could provide data for human fertility studies, child-spacing studies, and even more meaningful statistics on birth injuries, particularly when correlated with more detailed studies.

Mr. Schachter serves as one of a small group of statistical consultants to the five-year study of 40,000 pregnant women being made through sixteen teaching hospitals by a staff at the National Institute of Health.

This study is primarily to find the cause of cerebral palsy, mental retardation and other birth injuries. However, additional statistical data are being amassed on the mothers—time of having first child, number of children borne, difference in ages and the use of contraceptives.

Such studies could be made cooperative with birth certificate studies, even going back to the reporting physicians for further details if indicated.

Mr. Schachter pointed out that this country's birth registration was 99 per cent complete. He said that the vital statistics system offered the most efficient way to get accurate data. It is the population, and not just a sampling. It uses a permanent system and not a comparatively costly questionnaire.

The portion of the birth certificate dealing with congenital malformations, illegitimacy, etc., is reserved "for medical and health use only" and is never photographed for such purposes as getting a passport.

A recent Canadian study stated that about 40 per cent of the stillbirths, infant deaths and early defects, considered collectively, were believed to be hereditary in nature. It said: "Thus, appropriate population studies will permit very substantial numbers of families to be used in the detection of fertility differentials."—BESS FURMAN, *N. Y. Times,* October 18, 1959. *Reprinted by permission.*

NORMAL AND YELLOW LUPINE

Figure 10

A—Normal sweet yellow lupine var. Weiko III. *B*—Macro-1 mutant (MIM) from sweet yellow lupine var. Weiko III. Note abnormal leaves, prolific decumbent type growth.

A MACROMUTATION IN YELLOW LUPINE

(*Lupinus luteus* L.)

J. R. EDWARDSON AND M. K. CORBETT*

MUTATIONS which drastically alter the phenotype of yellow lupine have been reported by Kress[5] and Van Steveninck[13]. The mutation described by Kress exhibited subpetioled leaflets of tubular shape and bore deformed flowers which were completely sterile. Van Steveninck's mutant, apparently identical to that of Kress, exhibited some leaflets completely lacking lamina, anthers were reduced or absent and in some cases the style was branched or protruded from the side of the ovary. The *laciniata* mutant, studied by Lamprecht[6], in peas (*Pisum sativum* L.) closely resembles these mutations in yellow lupine.

A mutant similar to those reported by Kress and Van Steveninck was discovered in a seedling population of the sweet yellow lupine variety Weiko III. The present report concerns a detailed description of the mutant phenotype, the exclusion of virus infection as its inducing agent, and preliminary information about the inheritance of this mutation.

While testing approximately 15,000 plants of sweet yellow lupine var. Weiko III for virus transmission through the seed, a mutant seedling was observed. This seedling was transplanted to a pot of composted soil and designated macro-1 mutant (M1M). The mutant was grown under comparable conditions with normal plants of the Weiko III variety, in a screened greenhouse which was periodically sprayed with parathion.

Description of the Macro-1 Mutant

The leaves of normal yellow lupine plants (Figure 10*A*) are palmately compound with five to nine leaflets. The lower leaflets are narrow-obovate and the upper leaflets linear-lanceollate to oblanceollate. Leaflet laminae of the M1M plant were greatly reduced (Figure 11). The laminae varied from none to ¾ of that contained by normal leaflets. The basal margins of the leaflet laminae on the M1M plant were often fused imparting to the leaflet the appearance of a funnel. The length of the midrib varied from ½ to 2 times normal. Stipules on branch and leaf bases rarely developed and when present were never paired.

Cotyledons of the M1M plant were narrower, thicker and had smooth upper surfaces in contrast to the rough surfaces of normal cotyledons.

All primary leaves of the M1M plant abscised prematurely and branches developed from each axillary bud. These branches developed secondary leaves with petioles longer than normal. Leaf abscission also occurred on the secondary branches. All branches terminated in a raceme. The M1M plant exhibited a decumbent type of growth (Figure 10*B*).

The M1M plant produced a total of 80 racemes at maturity as contrasted with an average of 16 racemes on 12 comparable normal plants. Racemes and individual flowers of the M1M plant were smaller than those of normal yellow lupine.

Main racemes of a normal Weiko III plant and the M1M plant are illustrated in Figure 12. The enlargement at the right is a single flower from the M1M plant. All parts of the mutant flower exhibited many deviations from the normal. Bracts and paired stipules were present but greatly reduced. The calyx was divided into five short strap shaped parts while the normal calyx has an abaxial trifid apex and an adaxial bifid apex. The parts of the enlarged mutant flower are from left to right in Figure 12: divided wing, divided keel, androecium and gynoecium, deeply cleft standard and divided wing. The mutant keel does not enclose the androecium and gynoecium as does the normal keel. Filaments of

*Assistant Agronomist and Assistant Plant Pathologist, respectively. Florida Agricultural Experiment Station Journal Series No. 853.

MUTANT LUPINE

Figure 11

Left—Leaf of macro-1 mutant plant—note reduction of leaf laminae, elongated mid-ribs and funnel effect of laminae. Right—Leaf of normal sweet yellow lupine var. Weiko III.

the mutant were of equal length; the normal arrangement is alternating long and short filaments, the long filaments bearing oblong anthers, the short filaments globular anthers. Anther locules of the mutant were reduced and their shape intermediate between oblong and globular when both locules were present. More than half the filaments carried no anthers or bore only single locules; dehiscence did not occur. The gynoecium contained a style, sparsely hairy stigma, an open ovary with some or all of the ovules transformed into reduced pistils whose stigmas were devoid of hair. Figure 12 "a" and "b" represent the supernumerary pistil arising from a transformed ovule and the true pistil, respectively. The standard is deeply cleft, lacking in pigmentation in interveinal areas and very hairy along the main vein on the dorsal side. In normal yellow lupine the standard is not cleft, coloration is uniform and no hair occurs on any of the corolla parts. The wings of the M1M plant are a deeper shade of yellow than the normal.

Attempts to Recover Virus from the Mutant

Plants of yellow lupine infected with strains of bean yellow mosaic virus exhibit various leaf deformations[1]. Therefore, tests for the presence of a virus were conducted as follows. Leaves of the M1M plant were triturated with a mortar and pestle and the expressed juice was used for inoculation by the Carborundum gauze pad method. Leaves to be inoculated were dusted with Carborundum, inoculated and rinsed with tap water. The following plants were inoculated[1]: *Lupinus albus* L., *L. angustifolius* L., *L. luteus* L., *Crotalaria spectabilis* Roth., *Cassia tora* L., *Glycine max* (L.) Merr. var. Dortch, *Phaseolus vulgaris* L. var. Bountiful, *Pisum sativum* L. vars. Alaska and Little Marvel, *Vicia faba* L., *Vigna sinensis* (L.) Endl. var. Blackeye, *Datura stramonium* L., *Nicotiana tabacum* L. var. Turkish, and *N. glutinosa* L.

Epidermal strips of M1M leaflets were examined for the presence of virus inclusion bodies using the staining technique described by McWhorter[8].

Reduction of leaf laminae and funnel shaped leaflets frequently occur on single leaves of plants infected with strains of bean yellow mosaic virus but rarely are all leaflets of a single leaf involved in this abnormality. Virus infected plants always exhibit a mottle or mosaic and the leaves tend to be erect. Mosaics or mottles and erectness of growth were not exhibited by the M1M plant during any stage of its development.

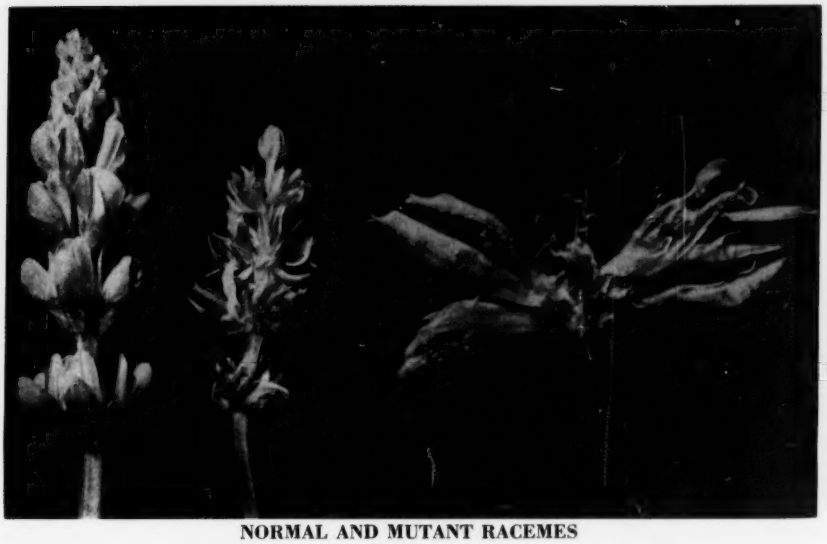
All tests for virus transmission from the M1M plant or progeny resulting from crosses involving the original M1M plant were negative. All tests for virus inclusion bodies were negative.

Inheritance

Attempts were made to self the M1M plant with pollen from crushed anthers and to cross the M1M plant with pollen from Weiko III and speckled seeded bitter strains of yellow lupine. Reciprocal crosses were also attempted. All crosses were made in the greenhouse. F_1 seed obtained from G463 × M1M and G465 × M1M crosses were planted in pots of composted soil and grown to maturity in a growth chamber during the summer of 1958. F_2 seed obtained from these plants are being grown in the greenhouse. All F_2 plants were tested, as described, for the presence of virus infection.

The macro-1 mutant plant was female-sterile. Pollen did not adhere well to the sparsely hairy mutant stigma. Natural self pollination probably would not occur because anthers of the M1M plant were never observed to dehisce and the structure of the keel would not allow the stigma to come in close contact with the anthers. It is doubtful that any normal ovules could have survived in the open ovaries of the M1M plant if fertilization had occurred.

Pollination of normal Weiko III and bitter yellow lupine varieties with pollen from crushed M1M anthers in most cases produced pods, with enlarged ovules, which abscissed within two to three weeks after pollination. Two viable seeds were obtained from crosses on G465 and one viable seed from crosses on



NORMAL AND MUTANT RACEMES

Figure 12

Left—Raceme from normal sweet yellow lupine var. Weiko III. Middle—Raceme from macro-1 mutant plant. Right—Enlarged single flower from raceme of macro-1 mutant; a—supernumerary pistil arising from a transformed ovule; b—true pistil. Flower parts—left to right—divided wing, divided keel, androecium and gynoecium, deeply cleft standard and divided wing.

G463. Both of these lines G463 and G465 are bitter and speckled seeded. The seed resulting from these crosses (G463 × M1M), (G465 × M1M) were normal in respect to size and shape and were speckled. The plants arising from these seed were also normal in all morphological aspects. Thus, there was no obvious effect of the mutation in the F_1. The growth of the three F_1 plants in the growth chamber during the summer of 1958 was poor. Two (G465 × M1M) F_1 plants produced a total of 41 seeds of which nine were normal with respect to size and shape and 32 were approximately 1/10 normal seed size. The one (G463 × M1M) F_1 plant produced no seed. Normal Weiko III plants grown under comparable conditions in the growth chamber also produced a high percentage of small seed.

The F_2 seeds were separated by size and planted in separate pots of vermiculite. The small seed did not germinate. The nine large (G465 × M1M) F_2 seeds germinated and were transplanted to pots of composted soil. The plants segregated 7 normal: 2 mutant phenotypes with all mutant characters occurring *en bloc*. At present the effect of the mutation on leaf abscission and flower characters in the F_2 has not been determined.

Discussion

None of the abnormal characters associated with the macro-1 phenotype are controlled by genes reported in the literature[4]. Van Steveninck[13] has described a phenotype occurring spontaneously in Weiko II sweet yellow lupine, and Kress[5] reported a phenotype in the X_2 generation of irradiated Gülzower sweet yellow lupine both of which seem to be identical to the macro-1 mutant (M1M). The descriptions in both reports agree in details with the appearance of the M1M plant with the following exceptions: 1) Van Steveninck states that leaves on lateral branches became complex by the branching of sub-petioles with each subpetiole carrying one to three leaflets. 2) Both authors reported their mutants were completely sterile. They do not indicate whether outcrosses were attempted; the macro-1 mutant would also be classified as completely sterile in the absence of outcrossing. Neither author described flower structure in detail nor mentioned leaf abcission. Van Steveninck also noted the similarity between aberrant leaflet types in his Weiko II mutant and those occurring in virus infected yellow lupines. He was unable to transmit a virus from the mutant to normal yellow lupine by means of aphids. He was able to induce occasional funnel leaflets in yellow lupine with 2-4-D treatments.

Funnel shaped leaflets have been observed in leaves of otherwise normal *L. luteus, L. angustifolius, L. mutabilis* Sweet and *L. pilosus* Murr. The funnel shaped leaflets in these species have not been associated with any of the

other abnormalities present in the macro-1 mutant. Only leaflets of single leaves are involved; thus, it is assumed that these funnel leaflets are the result of somatic mutations. Valid comparisons between this phenotype and that of the macro-1 mutant are not possible at present.

Weiko II, Gülzower, and Weiko III lupines in which similar macromutations have occurred possess common ancestry in von Sengbusch's strain 8 and strain 3535A[11, 12]. Whether this close relationship is significant in the occurrence of the mutations is unknown. That the relationship may have little or no significance is indicated by the work of Lamprecht,[6, 7] who described a *laciniata* mutant occurring in completely unrelated lines of *Pisum sativum* L. The *laciniata* mutant in many respects closely resembles the macromutants in yellow lupine.

The term macromutation is not applied to the macro-1 mutant as a synonym for "systemic mutation"[2] as Mayr[9] uses it, but it is used as defined by Goldschmidt[3]: "Such a study can give us a model for the action of a systemic mutation, if we find mutants, macromutants, which produce such a large deviation from normal that they show in their limited action upon a part of the body (but sometimes also the entire organism, e.g., corn grass) what can be accomplished within the frame work of viable development in regard to huge deviations, accomplishing in one step a real saltation of the kind we would expect to be produced by a systemic mutation." The appearance of the original mutant phenotypic complex and its transmission *en bloc* in the F_2, strongly suggests that the M1M plant is a macromutation. The absence of stipules, shape of leaflets, number of racemes and modifications of flower parts which occur in the M1M plant are variations which could easily permit the assignment of the mutant to a different genus and species category.

At present we can only speculate on the possible mechanism involved in the production of the macro-1 mutant. Lamprecht's[6] investigations have shown that chromosome aberrations are not necessarily involved in the production of macromutations but that their inheritance in *Pisum* and *Phaseolus* is controlled by single recessive genes. A deletion of linked genes could account for the appearance of the mutant. However, the deletion of large segments of any yellow lupine chromosome would be difficult to detect[10]. A type of chain reaction mentioned by Tedin and Hagberg[10] as possibly inducing three linked mutations in a single yellow lupine plant might also be responsible for the macro-1 mutant phenotype.

The absence of any effect in the F_1 indicates that the mutation is recessive. The small number of F_2 offspring does not permit assumptions about segregation ratios at present. It is anticipated that studies of F_3 and possibly backcross populations together with cytological studies of meiosis in heterozygotes will lead to an understanding of the mechanism of the origin and inheritance of the macro-1 mutant.

Summary

A mutant was discovered in a seedling population of sweet yellow lupine variety Weiko III. The mutant was highly sterile, the flowers were aberrant in all their parts, petioles lacked stipules and many leaves bore funnel shaped leaflets. The mutant plant exhibited many morphological characters which were so dissimilar to the normal lupine that it was classed as a macromutation and termed macro-1 mutant (M1M). All attempts to explain the mutant phenotype on the basis of virus infection were unsuccessful. Mechanisms which might control the mutant phenotype are mentioned. The mutation was recessive in the F_1. Segregation occurred in the F_2 population but the population was too small to permit conclusions concerning the inheritance of the macro-1 mutant.

Literature Cited

1. Corbett, M. K. A virus disease of lupines caused by bean yellow mosaic virus. *Phytopathology* 48: 86-91. 1958.

2. Goldschmidt, R. The Material Basis of Evolution. Yale University Press, New Haven. 1940.

3. ————. Theoretical Genetics. University of California Press, Berkeley. 1955.

4. Hackbarth, J. Die gene der Lupinenarten. *Zeitschrift für Pflanzenzüchtung* 37: 1-26. 1957.

5. Kress, H. Ergebnisse der Röntgenbestrahlung bei der Gülzower Süssen Gelblupine (*L. luteus*). *der Züchter* 23: 168-172. 1953.

6. Lamprecht, H. Durch Komplexmutation bedingte Sterilität und ihre Vererbung. *Archiv der Julius Klaus-Stiftung für Vererbungsforschung* 20: 126-141. 1945.

7. ————. Neues Auftreten der Mutation im interspezifischen Gen *Lac* von *Pisum*. *Agri hortique genetica* 14: 177-184. 1956.

8. McWhorter, F. P. Chlorazol fast pink B and trypan blue tests for virus and other proteinaceous inclusions. *Stain Technology* 32: 134-138. 1957.

9. Mayr, E. "Change of Genetic Environment and Evolution" Evolution as a Process, 157-180. G. Allen and Unwin Ltd. London. 1954.

10. Tedin, O. and A. Hagberg. Studies on X-ray induced mutations in *Lupinus luteus* L. *Hereditas* 38: 267-296. 1952.

11. Troll, H. L. Erbgang der Samenfarbe bei weisskörnigen gelben Süsslupinen (*Lupinus luteus*) und ihre Bedeutung für die Erhaltungszucht im Zussamenhang mit Weichschaligkeit und Leistungsfähigkeit. *der Züchter* 26: 41-53. 1956.

12. ————. Leistung von Wachstumsmutante aus Müncheberger Material von *Lupinus luteus*. *der Züchter* 28: 25-32. 1958.

13. Van Steveninck, R. F. M. The occurrence of funnel leaflets and ring fasciations in yellow lupines. *Annals of Botany* 20: 385-392. 1956.

IRREGULAR MOSAICISM IN A HEN

A. G. Cock*

IN mosaic individuals which have previously been described in the domestic fowl, the distribution of the two genetically different types of tissue within each individual has followed (with relatively minor irregularities) a well-defined pattern, in which the left half of the body is of one genotype and the right half of another. More than 20 such half-and-half mosaics have been described by various authors (see Cock[3] for a review). Among pigeons on the other hand, although two half-and-half cases have been described[7], most of the mosaics reported have had a quite irregular "patchwork" arrangement of the two genotypes. One genotype (the "normal") predominates, and the other is represented by one or more patches of tissue of irregular shape and position. Many of these irregular mosaics may be regarded as the result of somatic mutation (the term being understood to include chromosomal aberrations) occurring at a relatively late stage of development, but Hollander[8] has described seven mosaic pigeons for which such an explanation is more or less decisively excluded. In each of these the aberrant patches of tissue appear to be of purely paternal origin.

Irregular mosaics have not been unknown in the fowl [9,10] but the few cases reported can all be explained by somatic mutation, and no clear distinction can be drawn between such occasional cases and the ticking effects[2] which are a constant feature of fowls heterozygous at certain plumage-pattern loci. The case now to be described appears to be of similar origin to Hollander's pigeon mosaics.

The mosaic individual (♀ 4962/5R—see Figure 13) is an F_1 between two highly inbred lines: M (Rhode Island Red) ♂ × C (White Leghorn) ♀. It was noted when hatched to have had several chestnut patches in the down, and the adult plumage is an irregular patchwork of two types: (a) white with occasional blue-grey ticks, and (b) the chestnut-and-black pattern characteristic of the Rhode Island Red (R.I.R.) breed. Type (a) predominates, and this is the

RHODE ISLAND RED-WHITE LEGHORN MOSIAC

Figure 13

♀ 4962/5R, photographed in October, 1955. Note the widely scattered patches of R.I.R.-patterned plumage. There are also a few small blue-grey ticks; these are not distinct from the R.I.R. in this photograph, but see B7 of Figure 14.

*Agricultural Research Council Poultry Research Center, Edinburgh, 9, Scotland. Thanks are due to the National Poultry Institute Advisory Committee, Northern Sub-committee (now the Northern Poultry Committee of the Agricultural Research Council) for providing this bird for study. The study and progeny tests were carried out at the Poultry Genetics Unit, School of Agriculture, Cambridge.

normal pigmentation type of an M × C F_1 ♀. The areas of R.I.R.-pattern are extremely discontinuous in distribution. There is a large patch on the right wing, two more on the saddle, and several smaller patches on the neck. Elsewhere there are numerous individually mosaic feathers, each partly white and partly R.I.R.-patterned. These are particularly frequent on the right leg and left breast, although these regions have hardly a single feather which is entirely R.I.R.-patterned. The only large areas which are completely free from small patches of R.I.R. pigmentation are the tail and part of the left wing. The broad outlines of this distribution have remained essentially unchanged through two annual moults, although there have been considerable changes in detail. The three largest areas of R.I.R. pattern are all on the right side of the body, but this is presumably fortuitous, since in no other respect does the distribution show any approach towards bilaterality. The contrast with half-and-half cases is striking, even when allowance is made for the departures from a strict half-and-half arrangement (especially in respect of plumage pigmentation) that occur among the latter.

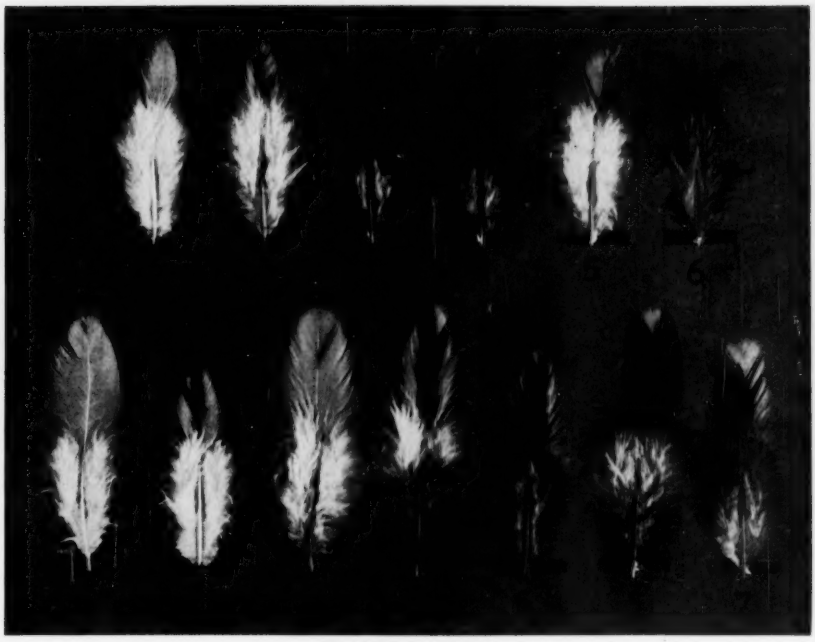
♀ 4962/5R was hatched in May, 1955 at the Northern Poultry Breeding Station, Reaseheath, Cheshire, England. It was first seen by the writer in September of that year, when it was brought to Cambridge. In every respect other than the pigmentation of the plumage it appears to have been a typical M × C F_1 ♀. It was a reasonably prolific layer, and remained in good health until killed in February, 1958. No internal abnormalities were found at autopsy, and the two sides of the body were not perceptibly different in size. Both parents were examined and found to be apparently typical members of their respective inbred lines. Eleven full-sisters of ♀ 4962/5R were examined; each was found to be a typical F_1, the plumage being white with blue-grey ticks, and entirely devoid of any red or brown pigment.

The C line of White Leghorns is known[4] to be homozygous for *E* (extended black) and *B* (barring, sex-linked) as well as for *I* (dominant white). The line was until recently segregating at the *Bl* (Andalusian blue) locus, but appears now to have become homozygous for *Bl*. The mother of ♀ 4962/5R was certainly homozygous *Bl/Bl*, as shown by the blue-ticked phenotype of all 11 normal daughters. All four of these genes are absent from the M line, so that a normal M × C F_1 ♀ would have the genotype *I/i; E/e; Bl/Bl; b/-*. It is thus highly improbable that the R.I.R.-patterned patches arose by somatic mutation or chromosomal aberration; this would re-

TABLE I. Frequencies of R.I.R.-patterned, mosaic and white feathers plucked from tattooed areas

		Entirely R.I.R.	Mosaic* 4	3	2	1	0	Entirely white	Total	No. follicles
Saddle	No. feathers	106	56	66	23	14	5	261	531	131
	%	20.0	10.7	12.2	4.3	2.6	0.9	49.2		
Breast	No. feathers	0	3	16	43	46	31	15	154	48
	%	0	1.9	10.4	27.9	29.9	20.1	9.7		

*Ticks of blue-grey are counted as white, since they are part of the normal F_1 phenotype. Mosaic feathers are graded according to an approximate assessment of the relative extent of R.I.R. and white areas within each feather:
grade 4 — < 2% white
grade 3 — > 2%, < 33% white
grade 2 — > 33%, < 67% white
grade 1 — > 67%, < 98% white
grade 0 — > 98% white



FEATHER TYPES

Figure 14

A—Six feathers grown in succession from the same follicle (saddle), ranging from entirely white (A 1) to entirely R.I.B. (A 6). Note the very small patches of white at the tips of A 3 and A 4. *B*—Selected mosaic feathers illustrating the characteristic types of arrangement of R.I.R. and white areas. B 1 and B 3 are from the breast, the remainder from the saddle. The series from B 1 to B 6 shows an increase in the extent of the R.I.R. area, while maintaining the essential arrangement of R.I.R. central stripe and white edges. B 7 is an exceptional feather containing, in different areas, three distinct types of melanocyte: (i) the white of the normal F_1; (ii) (on the left side of the vane) blue-grey—presumably derived from type (i) by loss of the *I* gene—and (iii) (as a central stripe) R.I.R.

quire the simultaneous loss of three genes (*I*, *E*, and *Bl*) which are, so far as is known, unlinked. Besides, the loss of these genes alone from the F_1 genotype would probably yield some other form of black-red pattern (e.g. that of the Brown Leghorn, or the black and pale golden-brown pattern of R.I.R. × Light Sussex ♀ ♀); further changes at one or more unidentified loci would be necessary to produce typical R.I.R. pattern. The pigmentation of the aberrant areas of ♀ 4962/5R is a faithful mimic of the paternal M line, even to the extent of reproducing a characteristic fault of the M line; irregular smudges of black in the vanes of saddle feathers. (In a "show-type" R.I.R. these are devoid of black). The only simple hypothesis that fits the facts is that the melanocytes of the aberrant areas are of purely paternal origin.

A progeny test of ♀ 4962/5R was carried out in 1956 by artificial insemination with a Brown Leghorn ♂ (*i*, *e*, *bl*). Thirty-seven chicks were hatched: 22 had white downs (*I*); six were black (*i*, *E*) and nine were brown-stripe pattern (*i*, *e*). Some of the black and brown-stripe chicks developed into blues (*E*, *Bl*) or blue-reds (*e*, *Bl*), others became black (*E*, *bl*) or black-red (*e*, *bl*). As far as they go, these results show that the ovary had the genotype to be ex-

pected from the pedigree of 4962/5R and from her "major" phenotype; they do not of course exclude the possibility that a relatively small part of her ovary had an aberrant genotype.

Problems of Cell-lineage

Although the genetic aspects of this mosaic are straightforward, some of the developmental implications are more puzzling. The distribution of R.I.R.-patterned patches in the plumage presumably reflects the distribution of descendants of one or more groups of cells of abnormal genotype in the neural crest—melanoblast—melanocyte series. (It is of course probable that the mosaicism also involves other tissues and cell types; in the absence of suitable marker genes this is not detectable.) It may be as well to deal immediately with a possible objection to this presumption. Are the numerous individually 'mosaic' feathers necessarily true melanocyte-mosaics? Might they not be populated entirely by aberrant melanocytes, the white in them being due to a gene such as *mo* (mottling—see Hutt[9], p. 215)? This would necessitate abandoning the hypothesis that the aberrant melanocytes are of purely paternal origin; *mo* (or any other gene with similar effects) is certainly not present in the M line, although it could be present in the C line, where it would be masked by *I*. There are, moreover, occasional feathers where one of the blue-grey ticks of the normal F_1 phenotype happens to occur on a feather which is partly R.I.R.-patterned; an example is shown in B.7 of Figure 14. In these cases at least, the restriction of R.I.R.-pigmentation to part of the feather must be due to melanocyte mosaicism, and not the presence of a mottling gene.

There are two ways in which cells of paternal genotype might become incorporated into the embryo. In some of the half-and-half mosaics, one half of the body seems to be of purely paternal origin[3,6]. This requires (a) two nuclei near the center of the uncleaved blastodisc (one of normal origin, the other perhaps derived by fusion of the nuclei of two supernumerary spermatozoa) and (b) a first cleavage plane coinciding with the embryonic axis. There is no reliable evidence that the first cleavage plane bears a constant relation to the future embryonic axis, and (a) followed by a transverse or near-transverse first cleavage, could well give rise to an irregular pigmentation-mosaic. There is some disagreement as to the exact location of presumptive areas in the chick blastoderm[13], but it is likely that in such a situation the neural crest would include descendants of both of the two original nuclei. The second way is that suggested by Hollander[8] for his mosaic pigeons. It is normal for several supernumerary spermatozoa to enter the egg, and to undergo some mitotic division at the periphery of the blastoderm before eventually disintegrating[1,12]. As a rare exception (perhaps connected with a change from haploidy to diploidy) these periblast nuclei might survive and be incorporated into the embryo. In the present case, especially if it be assumed that several nests of periblast cells were incorporated, this second way is easier to reconcile with the highly discontinuous distribution of the aberrant areas.

Some of the peculiarities of the distribution of the two pigmentation types in the plumage are shown in Tables I and II. These are based on repeated pluckings from selected areas outlined by tatoo-marks in the skin, so that each feather can be assigned to a particular follicle. The most striking feature is the high proportion of individually mosaic feathers; in the breast 90 percent of the feathers are mosaic (and none are entirely R.I.R.). Even in the saddle (where the area was chosen to include part of each of the two large R.I.R. patches) 31 percent of the feathers are mosaic; in contrast to the breast, the higher grades of mosaic predominate.

Table II shows that, although there is a marked tendency for certain follicles to pro-

TABLE II. Feather-types produced by individual follicles in the saddle

No. of feathers produced	R.I.R. only	White only	Mosaic only	R.I.R. and mosaic	White and mosaic	R.I.R. mosaic & white	Total no. follicles
6	2	9	3	1	2	1	18
5	2	13	3	12	—	1	31
4	5	8	1	7	6	—	27
3	1	23	11	8	5	2	50
2	—	4	1	—	—	—	5
Total	10	57	19	28	13	4	131
Proportion of mosaic feathers	0	0	1.0	.53	.43	.53	
Mean grade* of mosaic feathers	—	—	3.1	3.3	1.8	2.3	

*See Table I

duce only entirely white, or only entirely R.I.R. feathers, the degree of stability of individual follicles is rather low. Nearly half of the follicles have produced more than one type of feather (almost all the "mosaic only" follicles produced mosaics of more than one grade), and a few follicles have yielded the full range from entirely white to entirely R.I.R. The feathers of one such follicle are shown in Figure 14*A*. This variation in feathers grown by the same follicle is understandable in terms of the conclusions drawn by Cock and Cohen[5] as to the nature of the melanoblast reservoir of a feather papilla. As there has been no general tendency for white to increase with time at the expense of R.I.R., it is more difficult to account for the fact that R.I.R. is markedly less stable than white. Of 42 saddle follicles which have produced at least one entirely R.I.R. feather, only 10 have been stable (i.e. produced no white or mosaic feathers), whereas in the case of white, 57 out of 74 follicles have been stable.

Even more difficult to explain is the fact that within mosaic feathers the configuration of the two pigmentation types follows a distinct pattern or series of patterns. Representative feathers illustrating this series are shown in Figure 14*B*. The basis of the whole series is a central longitudinal stripe of R.I.R., and white lateral edges. The stripe is often very irregular in outline, with finger-like projections which tend to follow the lines of the barbs. Towards the base it usually broadens to cover the full width of the feather but often it does not reach the extreme tip. The different types of mosaic feather depend mainly on the width of this central stripe; it may be narrow and discontinuous, so that in extreme cases (B1 of Figure 14) only a small fleck of R.I.R. is present, near, but usually not quite at, the tip. On the other hand it may be so broad as to cover practically the whole feather. Where only a very small amount of white is present, this usually forms a roughly triangular patch at the tip (B6, A3 and A4). The important point is that feathers with the reciprocal type of arrangement (white central stripe and R.I.R. edges) do not occur; not a single feather of this kind has been found among several hundred feathers plucked or examined *in situ*. The situation is in some ways analogous to that encountered in the structural mosaics of Lillie and Wang[11]. They found that the dorsal half of a breast papilla combined with the ventral half of a saddle papilla yielded feathers with a breast-type central stripe and saddle-type lateral edges (cf. B4 and B5) whereas the reciprocal combination yielded feathers with a saddle-type tip and breast-type base (cf. B6). However, Lillie and Wang's mosaics depend on an accurate division of the papilla into dorsal and ventral halves, and they involve two types of epidermis, not, as in the present case, two types of melanoblast. No explanation which could be accepted with any great confidence can therefore be drawn from this analogy, although the similarity of the configurations obtained does suggest that there is some common basis underlying the two phenomena.

The occurrence of numerous mosaic feathers in which one or other component occupies only a very small part (sometimes less than 1 percent) of the feather seems to imply that the number of melanoblasts involved in the pigmentation of a single feather is rather large. On the assumption that each melanoblast involved produces the same number of melanocytes, which in turn occupy the same extent of the feather, there would need to be more than 100 melanoblasts in order to account for these mosaic feathers. The underlying assumption is admittedly dubious, and this estimate may therefore be too high, but it is probably of the right order of magnitude.

Summary

A mosaic F_1 ♀ from a cross of two highly inbred lines (Rhode Island Red × White Leghorn) is described. Parts of the plumage have the pigmentation pattern of the R.I.R. breed; the remainder has the normal F_1 phenotype (white with occasional blue-grey ticks). In a progeny test the mosaic bred as a normal F_1.

The melanocytes of the R.I.R.-patterned parts of the plumage must lack at least three genes (*I*, *E* and *Bl*) derived from the White Leghorn. Somatic mutation or chromosome aberration is therefore an unlikely origin, and it is suggested that the aberrant patches arose by incorporation into the embryo of cells derived from one or more supernumerary spermatozoa.

The distribution of R.I.R.-patterned patches in the plumage is quite irregular and highly discontinuous; the number of individually mosaic feathers is very large. These individually mosaic feathers, which in some regions comprise as much as 90 percent of all feathers, conform to one basic arrangement, with a central R.I.R.-patterned stripe and white lateral edges. The reciprocal arrangement (white center and R.I.R. edges) never occurs. These aspects are discussed in relation to cell-lineage in the neural crest — melanoblast - melanocyte series. No satisfactory explanation of them has been reached.

Literature Cited

1. Blount, M. The early development of the pigeon's egg, with especial reference to polyspermy and the origin of the periblast nuclei. *Jour. Morph.* 20:1-64. 1909.

2. Cock, A. G. The interpretation of autosexing. *Jour. Genet.* 51:421-433. 1953.

3. ————. Half-and-half mosaics in the fowl. *Jour. Genet.* 53:49-80. 1955.

4. ————. Segregation of hypostatic color genes within inbred lines of chicken. *Poultry Sci.* 35:504-515. 1956.

5. ———— and J. Cohen. The melanoblast reservoir available to a feather papilla. *Jour. Embryol. & Exp. Morph.* (in press).

6. GREENWOOD, A. W. and J. S. S. BLYTH. Genetic and somatic aberrations in two asymmetrically marked fowls from sex-linked crosses. *Heredity* 5:215-231. 1951.
7. HOLLANDER, W. F. and L. J. COLE. Somatic mosaics in domestic pigeon. *Genetics* 25: 16-40. 1940.
8. ————. Bipaternity in pigeons. *Jour. Hered.* 40:271-277. 1949.
9. HUTT, F. B. Genetics of the Fowl, p. 478. McGraw-Hill Book Co. New York. 1949.
10. KNOX, C. W. Color chimaeras in the domestic fowl. *Jour. Hered.* 22:133-134. 1931.
11. LILLIE, F. R. and R. WANG. The production and analysis of feather-chimaerae in the fowl. *Physiol. Zool.* 16:1-21. 1943.
12. PATTERSON, J. T. Studies on the early development of the hen's egg. I. History of the early cleavage and of the accessory cleavage. *Jour. Morph.* 21:101-134. 1910.
13. WADDINGTON, C. H. Principles of Embryology, p. 159. Allen & Unwin. London.

◆

A Case of Twin Embryos of the Guinea Fowl

JOSEPH C. DANIEL, JR.*

"IN birds there is apparently no special genetic tendency toward one-egg twinning for twinning is extremely rare"[6]. Several cases of monozygotic chicks have been recorded [2,3,5,6] and the causes of avian twinning adequately discussed,[6,7,8,9] but this rare phenomenon has not yet been reported for other birds, with the exception of doves[7] and possibly ducklings[4]. This note describes a case of one-egg twinning in the Guinea Fowl (*Numida meleagris*).

This twin pair (Figure 15) was discovered when being studied as part of an embryonic series. One twin was essentially normal for a typical eight somite (about 30 hours old) embryo. Its "autosite-parasite"[6] shows suppressed development and severe malformations, especially of the neural tube. Both embryos possess the primary optic vesicles and it is therefore assumed that they are of the same approximate age, even though the abnormal twin possesses only four distinct somites.

The specimens were sectioned for further study. The three germ layers are continuous from one twin to the other and the extra-embryonic coeloms of the two are confluent in the area immediately anterior to the somites but seem to collapse and become occluded with cells beyond that point. The endoderm of the mid-gut region is unbounded from one to the other and the foregut is considerably shorter in the abnormal twin. The right omphalomesenteric vein of the normal twin is directly connected with the left heart primordium of the abnormal partner, necessitating a sharing of the blood supply. The young age of the embryos makes it impossible to detect their sex but the nuclei of the mesenchymatous cells of both embryos show the presence of the sex chromatin and presumably establishes both twins as being male[1].

TWIN EMBRYOS
Figure 15

The twin on the left is a normal 30-hour old embryo. Its "autosite-parasite," on the right, is underdeveloped with severe malformations.

(*Continued on page* 192)

*Adams State College of Colorado, Alamosa. The author is indebted to Mr. Walt Money for providing the Guinea eggs from his flock and to Mr. Justus Dunlap for photographic assistance.

CYTOLOGICAL AND GENETIC CHANGES INDUCED BY VEGETABLE OILS IN TRITICUM

M. S. SWAMINATHAN AND A. T. NATARAJAN*

DURING the course of the mutation experiments carried out at this Institute in several crop plants, the earlier findings of Gustafsson[8] and other workers that there are marked differences in sensitivity to radiations among different plant species were confirmed. It was found that some oilseed plants like linseed and mustard are much less sensitive to radiation with X-rays and β-particles from P^{32} and S^{35} than cereals such as wheat and paddy. It seemed likely that the presence of oils in seeds might have a buffering effect on radiation and in an experiment designed to elicit information on this problem, seeds of *Triticum monococcum*, *T. aestivum*, *Oryza sativa* and *Vicia faba* were first soaked in some vegetable oils and edible fats and later subjected to X-irradiation. In this experiment, controls with no treatment and with seeds soaked in oils for various durations but not subsequently irradiated were kept. Observations on preparations of root tip mitosis made from this material showed that immersion of seeds in oil alone caused chromosome breakage in many cells[21]. Following this finding, a detailed study of the cytological and genetic effects of treatment with oils on *Triticum* species was undertaken. In an "*Allium* test" (Levan[11]) carried out during the earlier study, oils extracted from peanut (*Arachis hypogaea*), castor (*Ricinus communis*) and mustard (*Brassica campestris* var. *toria*) had been found to induce the highest frequency of chromosome aberrations and these three oils were therefore included in the present experiment.

Materials and Methods

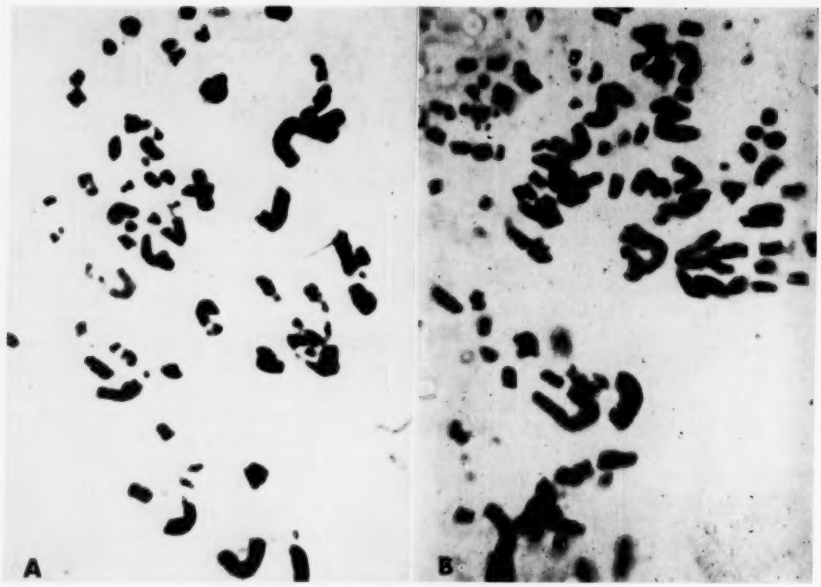
Dry seeds of einkorn (*T. monococcum* var. Japanese Early; $2n = 14$), emmer (*T. dicoccum*, var. Khapli; $2n = 28$) and bread wheat (*T. aestivum* var. C. 591; $2n = 42$) were soaked during the crop season of 1956, in peanut, mustard and castor oils extracted at the Division of Chemistry of this Institute. Seeds of einkorn and emmer wheats were soaked for six hours and the treatment lasted for 24 hours in bread wheat. At the end of the treatment, the seeds were wiped well with a muslin cloth and sown in the field. A few seeds were germinated in petri dishes for the study of root tip mitosis. The root tips were fixed for 24 hours in acetic-alcohol (1:3) and were stained in leucobasic fuchsin after acid hydrolysis. Microsporogenesis was studied in Feulgen squashes. Preparations were also made from control plants raised from seeds sown either dry or after presoaking in water. Seeds of all treated plants were collected separately and the individual plant progenies were raised during 1957. Morphological observations were recorded both in the year of treatment and during the second generation.

Results

Germination of seeds

The percentage of germination in different treatments is given in Table I. Control seeds showed 96 to 100 percent germination. From the data, it is seen that in *T. monococcum* and *T. aestivum*, treatment with peanut oil caused the greatest reduction in germination. The

*Cytogeneticist and Assistant Cytogeneticist respectively, Indian Agricultural Research Institute, New Delhi-12, India. The authors are grateful to Drs. B. P. Pal and S. M. Sikka for their advice and interest in the study. They are also indebted to Dr. E. R. Sears of the Missouri Agricultural Experiment Station and Dr. J. MacKey of the Swedish Seed Association, Svalöf, for their kind help in interpreting the data relating to the awn mutation.



BROKEN CHROMOSOME FRAGMENTS

Figure 16

Extensive fragmentation induced by oils in root tip cells. *A*—*T monococcum*, after two hours of treatment in mustard oil. *B*—*T. aestivum*, after six hours of treatment in castor oil.

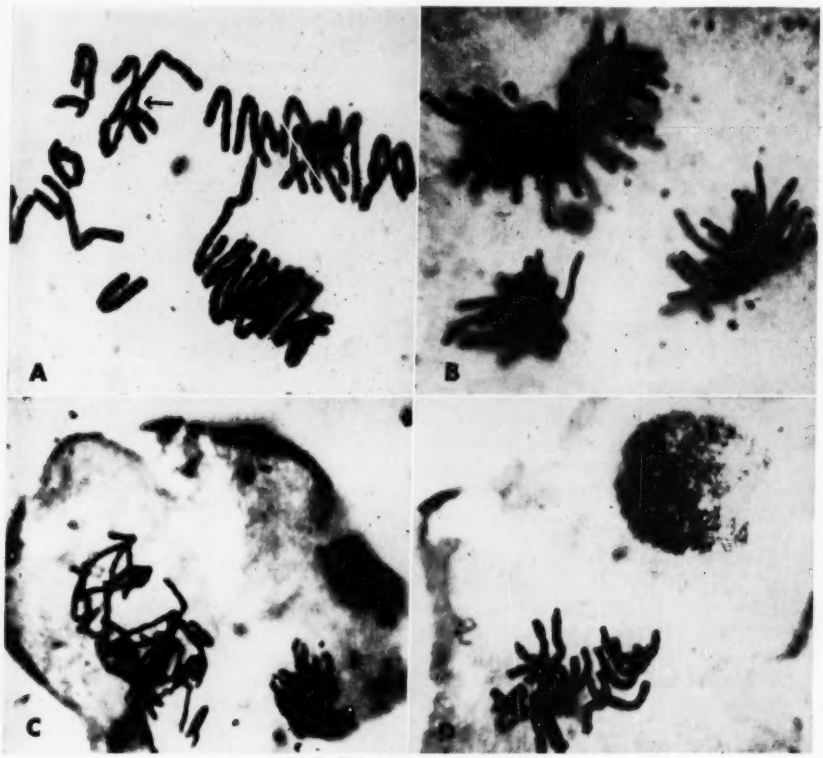
inhibition of germination was complete in peanut oil treated *T. monococcum*. The results also show that with increasing ploidy there is an enhanced survival. *T. aestivum* could stand a 24 hour treatment while in the diploid and tetraploid species even a six hour treatment was found to be fairly drastic. The trend of the relationship between polyploidy and sensitivity to oil treatments is thus similar to that found when ionizing radiations or other chemical mutagens are used[20].

Mitotic aberrations

In preparations made 24, 48 and 72 hours after germination, several chromosome aberrations were observed. Both chromatid and chromosome breaks were seen at metaphase and in some cells there was extensive fragmentation (Figure 16 *A* and *B*). Following treatment for six hours, the mean number of breaks per cell in *T. monococcum* was 0.882 in the case of castor oil and 0.647 in mustard oil. Treatment for one hour in peanut oil produced 1.28 breaks per cell. A striking feature of the metaphase plates in all the treatments was the absence of any evidence of reunion among broken fragments. In some chromosomes, the chromatids at the region of a break were asymmetrical, thus indicating the possibility that they were separately and independently broken. These observations would suggest that breakage occurs near

TABLE I. Percentage of germination of seeds in different treatments

Material	Duration of treatment	Percentage of germination in		
		Peanut oil	Castor oil	Mustard oil
T. monococcum	6 hrs.	0	12	8
T. dicoccum	6 hrs.	38	6	20
T. aestivum	24 hrs.	46	69	58



MUSTARD OIL TREATMENT

Figure 17

Abnormalities in root tip cells of *T. monococcum* treated with mustard oil for two hours. *A*—Anaphase with an 'error' configuration (arrow); *B*—tripolar spindle; *C* and *D*—binucleate cells with non-synchronised division.

the time of reduplication and is spread over a period of time. At anaphase, "error" configurations similar to those observed by LaCour and Rutishauser[10] in endosperm cells of *Scilla sibirica* exposed to X-rays were found (Figure 17 *A*). The "error" bridge occurred only in mustard oil treated *T. monococcum*, in which this configuration recurred frequently. No point effects were noticed near the centromere and both intra-chromatid and inter-chromatid associations leading respectively to loop like structures and configurations with unequal arms distal to the point of union, were seen. The mechanism controlling the formation of such configurations is not clear and it is difficult to say whether sub-chromatid breaks are involved, as assumed for similar configurations by Swanson[23] and LaCour and Rutishauser[10] or whether the matrix maintains the connection between the full chromatid pieces that were associated prior to breakage, thereby giving the appearance of part-chromatid breaks, as suggested by Ostergren and Wakonig[16]. Lagging chromosomes or fragments also occurred frequently at anaphase.

No chromosome stickiness was induced by any of the treatments. In fact, treatment of seeds with some oils, particularly that of castor for two hours or less, caused a marked clarification of the karyotype[22]. In *T. monococcum*, a few tetraploid cells with $2n = 28$ occurred in

all the treatments. Binucleate cells were also present, arrested cytokinesis being probably responsible for these aberrations. Mitosis was not synchronized in some binucleate cells; in one cell one nucleus was in telophase and another in early anaphase (Figure 17*C*) and in another cell, one nucleus was in interphase and another in metaphase (Figure 17*D*). Other combinations such as interphase and prophase and interphase and anaphase also occurred. Spindle abnormalities such as tripolar (Figure 17*B*) and multipolar spindles were found in a few cells.

Meiosis in treated plants

Meiosis was studied during microsporogenesis in three to four plants of each species in every treatment. Meiosis was regular in the control plants and the abnormalities induced by the treatments are described below:

T. monococcum: No abnormality was found in plants raised from castor oil treated seeds. In a plant belonging to the mustard oil treatment, however, all the cells had six bivalents and two univalents (Figure 18*B*). The two univalents were unequal in size and appeared as rings. Caldecott and Smith[3] have described similar configurations in X-rayed barley and have termed them "pseudo iso-chromosomes" since they considered that such configurations arise as a result of translocations between opposite arms of homologous chromosomes. The univalents observed in *T. monococcum* could also be pseudo iso-chromosomes of this description.

T. dicoccum: In some mustard and peanut oil treated plants, there were one quadrivalent and 12 bivalents at metaphase. The quadrivalents were of the chain or ring type. The frequency of chiasmata per nucleus (mean of 100 cells) was 35.4, 39.7, 37.8 and 37.9 in control, mustard oil, castor oil and peanut oil treatments respectively. A chromosome number other than 28 occurred in some microsporocytes and the frequency of their occurrence in the different treatments is given in Table II. Such "chromosome mosaic" cells (Frankhauser[7]), probably arise as a result of spindle abnormalities or chromosome non-disjunction and lagging during pre-meiotic mitosis. Anaphase I and subsequent stages were regular except for occasional irregular disjunction of the quadrivalent.

T. aestivum: Some cells at pachytene in a mustard oil treated plant showed deficiency-duplication configurations (Figure 18 *A*). At diakinesis and metaphase I, one quadrivalent and 19 bivalents were seen in many cells (Figure 18 *C*, *D* and *E*). The quadrivalents were either rings or simple chains. Occasionally, a trivalent and a univalent were present in the place of a quadrivalent. An unequal bivalent was found in a peanut oil treated plant. As in *T. diococcum*, there was no reduction in chiasma frequency in the treated plants, the mean frequency of chiasmata per cell (mean of 100 cells)

TABLE II. Frequency of PMC's with varying chromosome numbers in emmer and bread wheats

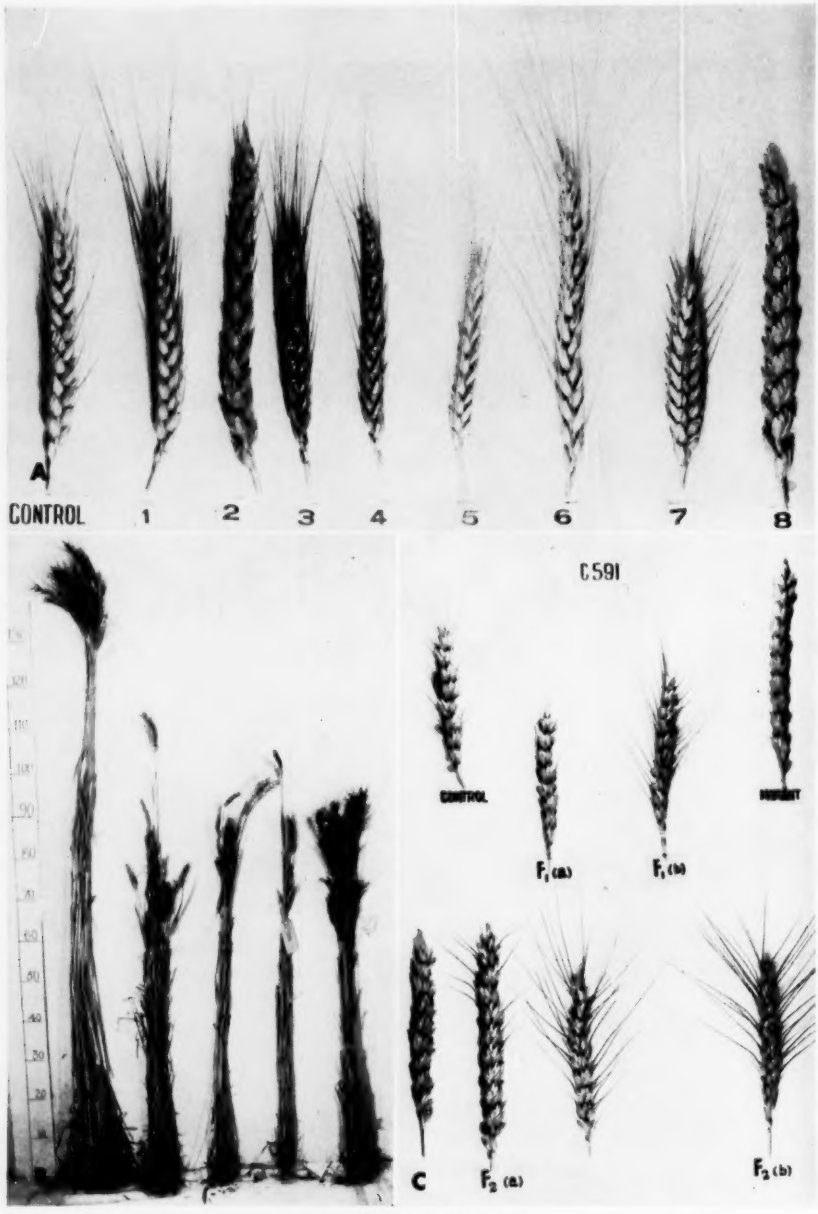
T. dicoccum										
Treatment	No. of pollen-mother-cells with $2n =$								Mosaic cells	
	12	14	18	20	26	27	28	34	Total	%
Castor oil	1	1	0	1	0	1	167	0	4	2.3
Mustard oil	1	1	0	1	0	2	173	0	5	2.8
Peanut oil	0	1	2	0	1	1	44	1	6	12.0

T. aestivum								
Treatment	No. of pollen-mother-cells with $2n =$						Mosaic cells	
	38	40	41	42	43	44	Total	%
Castor oil	3	3	0	70	1	1	8	10.3
Mustard oil	0	0	1	175	1	1	3	1.7
Peanut oil	3	31	2	206	2	1	39	15.8

MEIOSIS IN OIL TREATED PLANTS

Figure 18

A—T. aestivum, after 24 hours of treatment in peanut oil showing pachytene with deficiency-duplication configurations (arrow). *B—T. monococcum* after six hours of treatment in mustard oil, showing six bivalents and a pair of 'pseudo iso-chromosomes'. *C, D* and *E*—Metaphase I in *T. aestivum* treated with mustard, castor and peanut oils respectively showing one quadrivalent and 19 bivalents. *F—T. aestivum*, after 24 hours of treatment in peanut oil showing anaphase I with a dicentric bridge and an acentric fragment.



EARHEAD VARIATIONS

Figure 19

A—Earhead variations in the second generation plants of oil treatments, (C. 591). 1. Reduced awns in the lower spikelets (castor oil); 2. Long tipped (castor oil); 3. Red glume (peanut oil); 4. Red glume-speltoid (peanut oil); 5. Speltoid (mustard oil); 6. Lax (castor oil); 7. Dense spike of erectoid mutant (peanut oil); 8. Beardless mutant (peanut oil). *B*—Control (extreme left) and erectoid plants. *C*—Earheads of control, beardless mutant, two types of F_1 hybrids and the respective F_2 segregants, (C.591).

in control and peanut oil, castor oil and mustard oil treatments being 49.5, 50.8, 57.0 and 56.6 respectively. Chromosome mosaic cells occurred in *T. aestivum* also and the data are given in Table II. At anaphase I, laggards ranging from one to eight in number were seen. In mustard and peanut oil treatments, dicentric bridges and acentric fragments also occurred (Figure 18 *F*), indicating heterozygosity for inversions. The percentage of cells with laggards or bridges at anaphase I were 22.06, 35.9 and 25.3 in castor, mustard and peanut oil treatments respectively. Laggards were also seen in some cells at anaphase II and second division restitution leading to dyad fomation was observed in a few cells.

Pollen and seed fertility

A reduction of five to seven percent in pollen fertility (as measured by stainability in aceto-carmine) occurred in the treated plants of *T. monococcum* and *T. dicoccum*. In *T. aestivum*, a maximum reduction of nine percent occurred in a peanut oil treated plant. Seed setting in *T. aestivum* was decreased to the extent of 14.3, 22.5 and 24.6 percent in relation to the control in peanut, mustard and castor oil treatments respectively. Fifteen to 20 percent reduction in seed setting occurred in *T. dococcum* also. Practically no reduction in seed setting occurred in *T. monococcum*. It seems likely that in this diploid species, a strong intra-somatic selection may operate in the early stages following treatment which causes the elimination of a majority of affected cells.

Morphological changes observed in the first generation: Except for a little stunted growth in the early stages, no other abnormality was noticed in einkorn and emmer wheats in the year of treatment. In bread wheat, on the other hand, several changes relating to earhead characters were observed. C. 591, the variety of bread wheat used, is a very stable and homogeneous variety characterized by a fully bearded earhead, white and pubescent glumes, amber colored grains and medium maturity. No spontaneous mutation has been observed in the large control material of this variety grown each season during the past four years. Among the changes observed in the year of treatment were speltoid, sub-compactoid and lax earheads. In addition, one plant in the peanut oil treated material had completely beardless earheads. In all other characters this plant resembled C-591. The frequency of different first generation changes induced by the three oils is given in Table III. Each plant was harvested separately for raising the second generation. Crosses were made between the awnless plant and normal C-591 with the former as pistillate parent.

Mutations observed in the second generation: The individual plant families of einkorn, emmer and bread wheats were grown in separate rows and carefully screened for the occurrence of mutations.

TABLE III. Changes observed in the first generation in bread wheat

Treatment	No. of plants examined	Total no. of spikes	No of normal spikes	No. of spikes of type				Percentage of affected spikes
				Speltoid	Sub-compactoid	Lax	Awnless	
Peanut oil	46	323	299	20	0	1	3	7.42
Castor oil	69	326	319	7	0	0	0	2.07
Mustard oil	58	383	363	18	1	0	0	4.92

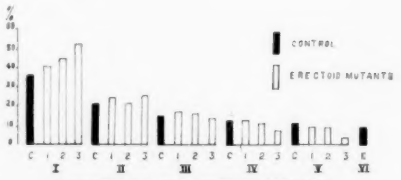
TABLE IV. Mutations observed in the second generation in bread wheat

Treatment	No. of plant progenies examined	No. of mutants of type								Mutations	
		Erectoid	Early	Dwarf	Red glume	Speltoid	Com-pactoid	Awn characters	Others *	Total no.	Per plant progeny (%)
Peanut oil	29	9	0	1	6	24	1	2	1	44	155.5
Castor oil	54	0	4	0	0	22	0	3	4	33	61.1
Mustard oil	55	0	0	0	0	1	0	1	0	2	3.6

*Includes color and hairiness of glumes, grass clumps and lax earheads.

A large control population was also raised. No phenotypically detectable mutation occurred in the progenies of einkorn and emmer wheats. There were, however, many prominent mutations in bread wheat (Figure 19 *A*) and the frequency of their occurrence is given in Table IV. While mutations affecting earhead characters were the most predominant, some early, dwarf, grass clump and erectoides mutations also occurred. The erectoides mutation was characterized by a short and stiff straw and compressed earhead (Figure 19 *B*). Both control and erectoid mutants had on an average 23 spikelets per earhead; the mean length of the earhead was, however, only 8.4 cms in the erectoid plants in comparison with 10.4 cms. found in the control. The variety C. 591 had on an average six internodes while none of the erectoid plants had more than five internodes. The relative internode lengths in the control and erectoid plants are given in Figure 20. The study of the cross-section area in the middle region of each internode revealed a significant increase in area in the lower three internodes of the erectoid plants. The erectoid mutation was thus characterized by (1) a decrease in the number of internodes; (2) a relatively shorter basal internode and a longer uppermost internode and (3) an increased cross-section area in the basal and middle internodes. These results are similar to the observations of von Wettstein (quoted by Ehrenberg *et al.*[6]), in erectoid mutations in barley and wheat.

Among speltoid mutants there were two types — one with glabrous glumes and red grains and another with the normal pubescent glumes and amber grains. In the peanut oil treatment, some mutants with red glumes occurred



LENGTHS OF INTERNODES
Figure 20

Histogram showing the relative lengths of internodes in control and erectoid mutants of C.591. Note that in the erectoid mutants there is an increase in the length of the uppermost internode (I) and a reduction in the length of the basal internole (V). The control has six internodes, while the erectoids have only five.

(Figure 19 *A*). In two plants, the red glumes were present only in some tillers, the other tillers having normal white glumes. The shade of glume color was lighter in such chimeras than in mutants in which all the tillers had red glumes. One long tipped mutant (awns to 2 cms. in length, present in the uppermost spikelets) as well as one mutant in which the awn length was reduced to half the normal length occurred in the castor oil progeny.

Genetics of beardless mutation

As mentioned earlier, a plant with completely beardless spikes occurred in the material raised from seeds soaked in peanut oil. There were four ear-bearing tillers in this plant. One spike was fixed for the study of microsporogenesis, one spike was crossed with a control C-591 plant and the remaining two spikes were selfed. Deficiency-duplication configurations at pachytene, heteromorphic bivalents at metaphase I and chromosome mosaic cells were some of the abnormalities found during meiosis in this plant. In the selfed progeny, beardless, long tipped and fully bearded

TABLE V. Segregation for awn character

Material	No. of plants observed		
	Beardless	Long tipped	Fully bearded
Control C. 591	—	—	all plants
Selfed progeny of beardless plant found in peanut oil treatment	22	14	5
F_1 beardless × control	0	2	2
F_2 from fully bearded F_1	0	0	170
F_2 from long tipped F_1	20	34	23

plants occurred. Among eight F_1 plants from the cross beardless $\times$ control, two were fully bearded, two were long tipped to half bearded (Figure 19 *C*) and four were grass clumps and did not flower. The fully bearded F_1 plants bred true, while the F_2 from the long tipped F_1 had fully bearded, long tipped and beardless plants in the ratio 1:2:1 (Figure 19 *C*). The data are given in Table V. Meiosis in the fully bearded, long tipped and beardless segregants also indicated that several changes such as inversions, interchanges and deletions had been transmitted to the progeny. The chromosome number was $2n = 42$ in all the plants studied except in a grass clump occurring in the selfed progeny which had $2n = 40$ in the root tips. A significant change associated with the loss of awns was a heavy reduction in tillering. Thus, while the normal C. 591 plants grown in the field had on an average 24.3 ear-bearing tillers (mean of 50 plants) the beardless C.591 mutants had only 13.4 tillers per plant (mean of 22 plants). The yield of grains per plant in the control and beardless mutant were 40.3 $\pm$ 2.13 grams and 16.4 $\pm$ 1.88 grams respectively.

Watkins and Ellerton[24] using conventional methods and Sears[18] by monosomic analysis have established that varieties of bread wheat possess a series of genes which either inhibit or promote the development of awns. The symbols commonly used to designate these genes are B_1 and B_2 for the dominant awn inhibitors present in chromosomes 9 and 10 respectively, *Hd* for a factor in chromosome 8 which reduces the awns and makes them curved and a_2, a_{13} and a_{20} for the awn producing genes, the suffix representing the chromosomes which have definitely been shown to have an awn promoting effect. The beardless C. 591 plant could thus have arisen as a result of a mutation leading to the origin of a dominant awn inhibitor or due to a deletion or mutation at the *"a"* locus. Based on the view[9] that an *"A"* gene is non-epistatic but incompletely dominant over an awn-producing allele *"a"*, a gene mutation at the *"a"* locus resulting in the loss of awn development would also be a dominant mutation. The segregation observed for awning in the F_1 as well as in the selfed progeny of the beardless plant would suggest one of the following possibilities. First, the beardless plant could itself be F_1 of a stray outcrossing between C.591 and a beardless wheat variety. This possibility seems unlikely since in crosses between all the bearded and beardless wheats in our collection, the F_1 shows some tipping and is not completely beardless. Also, the beardless plant had all the other characteristics of C.591 and showed segregation only for awning upon selfing. Secondly, the initial beardless plant could have had a chimerical composition with the germinal layer, giving rise to the glumes being homozygous for the mutation and the layer giving rise to the micro- and mega-sporocytes being heterozygous for it. This explanation may be satisfactory for a single earhead but is very unlikely for the whole plant since three to four ear primordia are already differentiated in the treated seed. Also, for one cell to become homozygous for the mutation it would be necessary that there be a non-disjunction of the chromosome concerned and at the same time a non-disjunction to the other pole of the homologous chromosome carrying the non-mutated allele. This cell would then have had to become the only surviving cell in the entire germinal layer concerned. The third possibility is that the particular combination of deficiencies and other chromosome structural changes found in the beardless plant may have had a modifying effect so as to make the heterozygous mutant plant fully beardless. Some such reason may also account for the excess of beardless plants in the selfed progeny of the mutant (Table V).

While it is thus difficult to offer a convincing explanation for the observed results, it is of interest that the F_1 of the cross beardless $\times$ control was either fully or half bearded. This would rule out the possibility that dominant epistatic factors are involved in the mutation to the beardless condition. It seems more likely that the beardless mutation owes its origin to the deletion of the awn

producing genes present in C. 591. The data in Table V would indicate that the awn promoting effect of chromosomes 2, 13 and 20 is due to single genes. The differences in awn expression observed between homozygous and heterozygous genotypes in the C. 591 cross would suggest that the awn producing alleles are neither wholly dominant nor wholly recessive. However, from the observation that, in the absence of inhibitory factors, awn development is permitted even in heterozygous genotypes, it would be more logical to refer to awn producing genes by dominant symbols.

Discussion

The results of the present study clearly establish that some vegetable oils can induce both chromosome breakage and viable mutations in wheat. These two properties of the oils may be correlated since many viable mutations in bread wheat arise from part or whole chromosome deficiencies or duplications[12]. It is particularly interesting that while chemical mutagens like nitrogen mustard have not been useful for inducing viable mutations in bread wheat[14], agents like peanut oil are very effective. In fact, among various mutagens like X-rays, fast neutrons, P^{32}, S^{35}, nitrogen mustard and different oils used by us to induce mutations in the variety C.591, peanut oil gave the highest mutation rate per plant progeny[17]. The maximum mutation rate per plant progeny obtained by MacKey[12] in the bread wheat variety Rival was 167 percent using fast neutrons at the dose of 16,800 *dis* units. In our experiments in C. 591, peanut oil gave a mutation rate of 155.5 percent and the mutagen which gave the next rate was S^{35} (94 percent). In contrast to the high mutation rate observed in bread wheat following treament with oils, no mutation was found in einkorn and emmer wheats. This further supports the conclusion of MacKey[13] that the polyploid state, far from being a handicap in mutation experiments as anticipated by Stadler[19], is really an advantage since it permits many chromosome structural changes.

From the present data it is difficult to say whether any differences exist in the spectrum of mutations yielded by the diffierent oils. The red glume color mutation as well as the erectoid mutation occurred only in peanut oil treatment. Using chlorophyll mutations as indices, Ehrenberg *et al.*[6] have demonstrated striking differences in the relative frequencies of different types of mutations induced by different chemical mutagens. The experiments with the different oil treatments would have to be repeated before it can be known whether certain types of mutations are always likely to be induced by a particular treatment.

The unsaturated fatty acid components probably form the mutagenic fraction of the vegetable oils. Auerbach and Robson[1] found that the essential oil of mustard (allyl-iso-thiocyanate) had mutagenic properties but they were doubtful whether it produced chromosome aberrations. It was subsequently reported by D'Amato and Avanzi[4] that allyl-iso-thiocyanate does not cause chromosome breakage. Studies in *Drosophila* have shown that sesame oil[5] and peanut oil[2] have no mutagenic properties. However, in the experiments of these authors, the oils were used to dissolve certain chemical mutagens and detailed studies on the effects of the oils themselves do not seem to have been undertaken. We are currently studying the mutagenic properties of the fractionated components of peanut, castor and mustard oils and the results may help to identify the mutagenic fraction.

A striking feature of the metaphase stage in the root tip cells, 24 to 72 hours after treatment with oils, was the absence of any evidence of reunion among broken fragments. There was extensive fragmentation in many cells but no evidence of reunion was found except in some cells which had "error" bridges at anaphase. The fact that in the same treatments, interchanges and inversions could be detected at meiosis suggests that a delayed reunion of broken chromosomes could have occurred. It is possible that treatment with oils causes chromosome breaks which remain open by some process analogous to that suggested by Wolff and Luippold[25] to explain the action on chromosomes of enzyme poisons like dinitrophenol.

An important indication provided by the study of awn expression in the cross beardless mutant $\times$ normal C. 591 is that in genotypes free of inhibitors, awn producing alleles show partial dominance in the heterozygous state. All beardless wheat varieties subjected to monosomic analysis have been found to possess one or more inhibitory factors. This and the occurrence of beardless or slightly tipped F_1 in nearly all reported crosses between beardless and bearded varieties would suggest that strains beardless solely due to the lack of awn producing genes are rare or probably even non-existent in bread wheat. This interesting evolutionary feature tends to become obscured when the awn producing genes are referred to by recessive symbols. Sears (personal communication) has expressed the view that deficiencies of awn promotors must have occurred in the history of wheat very much more frequently than have awn inhibitors. The reason why none of them has been retained must be that such deficiencies result in too great a reduction in yield. This inference is supported by our observation that the beardless C. 591 mutation is characterized by an appreciable reduction in the number of tillers and grain yield per plant. However, these plants have several chromosomal aberrations and it is difficult to be sure that the deletion of the awn producing allele alone is responsible for the reduced yield. Further studies on induced beardless mutations of fully bearded wheats

would help to elucidate this interesting evolutionary problem.

Finally, the cytological and genetical effects of the vegetable oils studied by us assume importance in view of the relationship between mutagenicity and carcinogenecity. A comparative survey of the results not only with radiations of different types but also with chemical mutagens suggests the view that the effects of these agents on genes and chromosomes form the basis of their effects in producing malignancies[15]. The role of nutrition with reference to the incidence of cancer is now widely realized and there are indications that a search for carcinogenic compounds in human dietary regimens might be worthwhile. Peanut and mustard oils are widely used as cooking media in tropical countries and it may be worthwhile pursuing the present line of work from this point of view.

Summary

Chromosome and chromatid breaks, "error" configurations, binucleate cells with or without synchronized mitosis and spindle abnormalities were observed in root tip cells of einkorn, emmer and bread wheats treated with peanut, mustard and castor oils. Though reunion following chromosome breakage appeared to be rare in mitotic plates, multivalent associations and inversion bridges were seen during microsporogenesis, thus indicating that delayed reunion may occur. Several morphological changes were observed during the year of treatment in bread wheat including a plant with completely beardless spikes. While no mutation was detected in einkorn and emmer wheats during the second generation, several viable mutations were isolated in bread wheat. Peanut oil yielded the maximum number of mutations among which the erectoides mutations may be of economic value. The genetics of the beardless mutation was followed and the results indicated that in the absence of epistatic genes, awn producing alleles are partially dominant. It seems likely that the rarity in nature of bread wheat varieties beardless due to the lack of awn producing genes, arises from a heavy reduction in yield which the deletion of such genes causes.

Literature Cited

1. AUERBACH, C. and J. M. ROBSON. Production of mutations by allyl-iso-thiocyanate. *Nature* 154:81. 1944.

2. BIRD, M. J. Chemical mutagenesis. *Drosophila Information Service* 25:100. 1951

3. CALDECOTT, R. S. and L. SMITH. A study of X-ray induced chromosomal aberrations in barley. *Cytologia* 17:224-242. 1952.

4. D'AMATO, F. and M. G. AVANZI. Studio comparitivo dell' atti vita citologica di alcume essenzi. *Caryologia* 1:175-193. 1949.

5. DEMEREC, M. Induction of mutations in Drosophila by dibenzanthracene. *Genetics* 33: 337-348. 1948.

6. EHRENBERG, L. A. GUSTAFSSON and D. VON WETTSTEIN. Studies on the mutation process in plants—regularities and intentional control. Conference on Chromosomes, Wageningen. 1-29. 1956.

7. FRANKHAUSER, G. The effects of changes in chromsome numbers on amphibian development. *Quart Rev. Biol.* 2:20-78. 1945.

8. GUSTAFSSON, A. The X-ray resistance of dormant seeds in some agricultural plants. *Hereditas* 30:165-178. 1944.

9. HEYNE, E. G. and R. W. LIVERS. Monosomic analysis of leaf rust reaction, awnedness, winter injury and seed color in Pawnee wheat. *Agron. Jour.* 45:54-58. 1953.

10. LACOUR, L. F. and A. RUTISHAUSER. X-ray breakage experiments with endosperm. *Chromosoma* 6:697-709. 1954.

11. LEVAN, A. The influence on chromosomes and mitosis of chemicals as studied by "Allium Test." Proc. 8th Int. Congr. Genet. *Hereditas Suppl.* 325-337. 1949.

12. MACKEY, J. Neutron and X-ray experiments in wheat and a revision of the speltoid problem. *Hereditas* 40:65-180. 1954.

13. ————. Mutation breeding in polyploid cereals. *Acta Agri. Scand.* 4:549-57. 1954.

14. ————. The biological action of mustards on dormant seeds of barley and wheat. *Acta Agri. Scand.* 4:419-29. 1954.

15. MULLER, H. J. The nature of the genetic effects produced by radiation. *Radiation Biology* 1:351-473. 1954.

16. ÖSTERGREN, G. and T. WAKONIG. True or apparent sub-chromatid breakage and the induction of labile states in cytological chromosome loci. *Bot. Notiser:* 357-375. 1954.

17. PAL, B. P., S. M. SIKKA, M. S. SWAMINATHAN and A. T. NATARAJAN. Frequency and types of mutations induced in bread wheat by some physical and chemical mutagens. *Wheat Information Service* 7: 1958.

18. SEARS, E. R. Cytogenetic studies in the polyploid species of wheat. II. Additional chromosome aberrations in *Triticum vulgare*. *Genetics* 29: 232-246. 1944.

19. STADLER, L. J. Chromosome number and mutation rate in *Avena* and *Triticum*. *Proc. Natl. Acad. Sci. U. S.* 15: 876-881. 1929.

20. SWAMINATHAN, M. S. Polyploid and sensitivity to mutagens. *Ind. Jour. Genet. & Pl. Breed.* 17: 296-304. 1957.

21. ————, and A. T. NATARAJAN. Chromosome breakage induced by vegetable oils and edible fats. *Curr. Sci.* 25: 382-384. 1956.

22. ———— and ————. Chromosome spreading induced by vegetable oils. *Stain Tech.* 32: 43-45. 1957.

23. SWANSON, C. P. The effect of infrared treatment on the production of X-ray induced changes in the chromosomes of *Tradescantia*. *Proc. Natl. Acad. Sci. U. S.* 35: 237-244. 1947.

24. WATKINS, A. E. and S. ELLERTON. Variation and genetics of awn in *Triticum*. *Jour. Genet.* 40: 243-270. 1940.

25. WOLFF, S. and H. E. LUIPPOLD. The production of two chemically different types of chromosomal breaks by ionizing radiations. *Proc. Natl. Acad. Sci. U. S.* 42: 510-514. 1956.

A MUTATION CAUSING CONGENITAL CLUBFOOT IN THE HOUSE MOUSE

M. W. ROBINS*

IT was long supposed that congenital clubfoot in man was caused at random by abnormal mechanical factors acting on the embryo *in utero*. This idea has been displaced by the conception that it is caused by genetic factors which may or may not act on the embryo in a direct mechanical manner. Twinning studies and the analysis of pedigrees have proved that in the majority of cases the defect is genetically controlled. Embryological and etiological studies on clubfooting are not common, perhaps due to the scarcity of material. Several mutant mouse strains possess features which are similar to inherited human pathological conditions, and clubfoot occurs in certain mutant strains of mice. But these do not provide suitable material for studies in parallel pathology owing to irregularity of manifestation, other major pleiotropic effects, etc. The present paper describes a new mutation of the mouse causing congenital clubbing of the hind feet and frequent unilateral and bilateral clubbing of the fore feet.

ADULT HOMOZYGOUS CLUBFOOT MOUSE

Figure 21

This *clcl* mouse shows clubbing of both hindfeet, with associated calousing, and mild clubbing of the right forefoot.

Origin

Late in 1954 a non-agouti female and an albino male, both of unknown ancestry, were mated and produced a litter of four. After weaning the two surviving mice, the mating was broken up. The survivors proved to be female and male, both pink-eyed non-agouti dilute; and they were mated together. In the first litter of this mating one clubfooted mouse was observed. Further litters born to the same mating contained similar animals, the ratio of normal to abnormal offspring being roughly 3:1. This ratio suggested that the character was inherited in a simple recessive manner. Further matings were therefore made up for the preservation and study of the character which was named clubfoot.

Description

Clubfoot mice are slightly lighter than normal sibs at birth, when they can be classified by the abnormal position in which their feet are held and their inability to plantar flex their hind feet.

*The work was completed at the Royal Veterinary College, London, where the author was employed by Dr. D. Michie and Dr. A. McLaren to whom he is grateful for facilities to complete this work and for their help and encouragement, and also to Dr. T. C. Carter for helpful criticism. The author's present address is Department of Zoology, University College, London W. C. 1.

Pre-weaning mortality is high; 59 percent of 157 clubfoot mice died before 14 days. Most of this is accounted for by deaths in the first two days. Many clubfoot mice are no doubt eliminated in the pre-weaning period by starvation due to intra-litter competition for milk. By 50 days a further 28 percent died, by 100 days 3 percent more had died. 9.5 percent survived over 100 days. The oldest clubfoot mouse lived 297 days: the oldest normal sib lived 599 days. Growth curves constructed for clubfoot mice and normal sibs show that the growth of the mutant animals is steady but takes place at a reduced rate. At all stages clubfoot mice are lighter in weight than normal sibs.

The clubbing is a simple calcaneum (dorsiflexed) type, always affecting both hind feet and with irregular unilateral and bilateral expression in the fore feet. Even when the fore feet are not clubbed, they are still abnormal, for the digits show the same flaccidity as those of clubbed feet. Owing to the abnormal position of the hind feet, the skin is often rubbed off the portion of the heel where the weight is supported. This has never been observed in clubbed fore feet, but soft fleshy pads of tissue have been observed on the weight-bearing regions of both fore and hind feet.

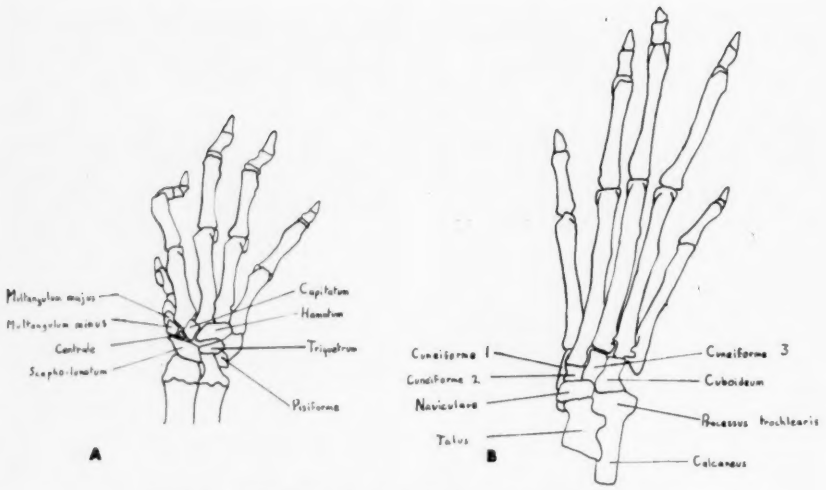
When a clubfoot mouse is held firmly, the clubbed feet can be moved manually, against a slight resistance, to the normal position. When released, the foot returns to its original position. Similar results occur under ether anesthesia. When the hind feet are forcibly plantar flexed, the skin on the dorsum of the foot appears to be in tension, thrown up in longitudinal ridges. Clubbed fore feet do not show this. Affected animals are able to plantar flex their feet, though only to a very slight extent. When pulled backwards by the tail, an action which causes normal mice to plantar flex strongly, clubfooted mice are unable to plantar flex to any degree which would diminish their slide. There is no control over the digits of any foot, whether clubbed or not. When suspended by the tail and offered a slender supporting object the mice are unable to grip it with their digits as normal mice do. Further evidence is seen in the normal position of the digits, which are splayed apart or held together in a degree which is directly dependent on the position and movement of the mouse. Lack of tenacity is in some degree compensated by the use of pincer movements of the limbs.

The eyes of some animals are frequently blocked by a viscous discharge which often gums the lids together. The area between the lids is often diminished, and in some cases the lids are periodically denuded of hair. This condition has never been observed in normal mice of the same strain. Most animals loop their tails forward over the back when walking, possibly to balance abnormal weight distribution.

Eight clubfoot mice have shown, in addition to the normal syndrome, dorsiflexion and eversion of the lower forelimbs. This abnormality has been observed unilaterally and bilaterally and in varying degrees of severity. The af-

TABLE I. Carpal and tarsal fusions in 15 pairs of clubfoot mice and normal sibs

	Types of fusion	Clubfoot Left	Clubfoot Right	Unaffected Left	Unaffected Right
Hamatum-capitatum-centrale	Hamatum-capitatum	7	5	0	0
		2	3	0	0
	Hamatum-capitatum-centrale-multangulum minus	4	3	0	0
	Capitatum-centrale-multangulum minus	0	1	0	0
	Capitatum-centrale	0	0	2	3
	Centrale multangulum minus	4	4	6	5
	Total:	17	16	8	8
	Cuneiform 3—naviculare	12	12	1	1
	Cuneiform 3—naviculare-cuneiform 2	3	3	0	0
	Cuboidenum-calcaneum	2	2	0	0
	Total:	17	17	1	1



NORMAL OSTEOLOGY OF THE FOOT OF THE MOUSE

Figure 22

A shows the structure of a normal forefoot and *B* shows the structure of a normal hindfoot of the mouse.

fected mice occurred in four matings which were not closely related.

42 matings involving 12 homozygous clubfoot mice were made up; six of these, three females and three males, proved fertile, producing 10 litters only. There has been a recent increase in the viability and fertility of homozygous mice but this is masked in the figures given by a preponderance of older data.

The heterozygotes are apparently normal in every way except for an abnormality due to genetic background which is mentioned below.

General Anatomy

Gross dissection of homozygous clubfoot mice and normal sib controls revealed no abnormality except that the muscles of the lower limbs show a diminution of muscular tissue. This occurs to such an extent that the separation of individual muscles is extremely difficult or impossible. Muscles controlling palmar and palmar flexion are present. The muscles of the upper limbs are apparently unaffected.

A small, uncontrolled series of five alizarin preparations of clubfoot mice was made for the study of bony abnormalities. They revealed that the only major abnormality was tarsal and carpal fusion. A larger series was therefore made for a more detailed study of skeletal abnormalities. The series consisted of 15 clubfoot mice and 15 normal litter mate controls, of age range from 14 to 173 days. Once again the only major skeletal abnormality was tarsal and carpal fusions. The observations on this abnormality are summarized in Table I. Fusions occur in both clubfoot and normal sibs. The fusions in club-

TABLE II. The single factor segregation of *cl*

Type of mating	Phenotype of progeny		
	±	*cl*	Total
± ± × *cl cl*	16	0	16
cl cl × *cl cl*	0	37	37
± *cl* × *cl cl*	6	4	10
± cl × ± *cl*	578	181	759

TABLE III. Linkage tests between *cl* and sex

Mating type (Mixed cross repulsion	Offspring ♂ +	♀ +	♂ *cl*	♀ *cl*	Total
cl Y .... *cl X* / + *Y* .... + *X*	56	68	26	17	167
$2 \times 2\chi^2 = 2.992$					

foot mice are more numerous and in some cases a greater number of bones are involved. Unilateral fusions showed no tendency to occur preferentially on the left or right side. As one type of fusion occurs only in the normal controls, and another type more frequently in normal controls, it is possible that these are caused by a strain abnormality as opposed to 'background effects' or heterozygous action of the clubfoot gene. On the present strain background the clubfoot gene increases the number and extent of tarsal and carpal fusions.

Genetics

Single factor segregation (Table II) shows that the mutation acts as a recessive gene of complete penetrance. The segregation of clubfoot and sex in repulsion crosses (Table III) shows that the gene is not sex-linked. The non-significant deviation which is observed can in any case be attributed to the sex-ratio disturbance among affected mice which is described below.

Two outcrosses of homozygous clubfoot males provided material for linkage tests with the recessive markers pink eye, *p*, dilute, *d*, brown, *b*, vestigial, *vt*, and waved *wa-2*. The figures collected (Table IV) indicate that *cl* is not closely linked with any of these genes.

An abnormal sex-ratio, 81♀ : 124♂ has been observed in the clubfoot mice. The abnormal sex-ratio is paralleled by the similar sex-ratio abnormality reported in surveys on human congenital clubfooting. The *clcl* mice were sexed at birth and checked at 10 days if they survived. To exclude the possibility that the whole strain produced more male offspring than females the sex-ratios of various groups within the colony were compiled. The results, (Table V) show that the only large diviation from the expected ratio occurs in *clcl* mice. Since the clubfoot condition has so gross and clear-cut an expression, the explanation for this disturbance of sex-ratio seems more likely to lie in defective pre-natal viability in clubfoot females than in misclassification of a proportion of such females as normal.

Discussion

Clubfooting appears in several mutant strains, but each of these is clearly differentiated from the mutation clubfoot. The recessive condition "myclencephalic blebs"[2] shows clubfoot of various types associated with syndactyly in its extensive syndrome. The most frequent type of clubbing is a dorsiflexion type and usually affects only the toes; very rarely are all feet affected. The condition shows irregular manifestation. The mutation or mutation-constellation, posterior reduplication[3] shows clubfoot of an unspecified type in its syndrome. Here the clubfoot is associated with polydactyly. Clubbing of the fore feet occurred in the syndrome of a recessive, sub-lethal mutation described by Kobozieff and Pomriaskinski-Kobozieff[4]. The clubbing was of a palmar

TABLE IV. Linkage tests with *cl* (I—intercross, C—coupling phase, R—repulsion phase)

Mutant *M-m*	Mating type	++	*m* +	+ *cl*	*m cl*	Total	R.F.	χ^2
P	IC	56	19	28	3	106	44.3	3.526
d	IC	45	34	13	6	98	47.7	2.000
se	IC	8	3	6	1	18	50.0	0.617
b	IC	55	23	13	5	96	37.5	0.105
b	IR	18	4	2	1	25	76.0	0.360
vt	IC	26	14	9	1	50	46.0	0.389
vt	IR	27	7	11	5	50	64.0	0.075
wa-2	IC	26	10	7	3	46	40.0	0.096
wa-2	IR	19	3	4	5	31	77.4	6.627

TABLE V. The sex ratios of clubfoot and normal mice. The totals do not tally exactly with the corresponding totals of Table II because some mice died and were lost before they were definitively sexed.

Type of mice	♀	♂	Total
cl cl mice from *cl cl* × *cl cl* matings	15	17	32
cl cl mice from ± *cl* × *cl* matings	1	3	4
cl cl mice from ± *cl* × ± *cl* matings	65	104	169
All *cl cl* mice sexed	81	124	205
Normal mice from ± *cl* × ± *cl* matings	206	239	445
Normal mice from ± ± × *cl cl* matings	6	9	15
Normal mice from other matings in the same stock	327	317	644
All normal mice sexed	539	565	1104

For the difference between all *cl cl* and all normal mice, $\chi^2 = 6.011$, $P > 0.02$.

flexion type.

The results described are consistent with the view that the major causative factor is atrophy of the limb muscles which result in clubbing of the feet arising in foetal life. Whether the atrophy is a primary defect of the muscular tissue or a secondary defect resulting from anomalies of the nervous system has not been determined.

Summary

An autosomal, recessive gene of complete penetrance causing congenital clubfoot with regular bilateral manifestation in the hind limbs and irregular unilateral and bilateral manifestation in the fore limbs, arose spontaneously in outbred mice. The gene was named clubfoot, symbol *cl*. The clubbing is in all cases a simple calcaneum type. The gene increases the number and distribution of tarsal and carpal fusions. Viability is low. The fertility of both sexes is low. There is a significantly displaced sex-ratio in affected animals, roughly 2♀ : 3♂. No close linkage exists between *cl* and sex, *p*, *d*, *se*, *b*, *vt* and *wa-2*.

Literature Cited

1. Böök, J. A. A contribution to the genetics of congenital clubfoot. *Hereditas* 34:289-300. 1948.

2. Bagg, H. J. Hereditary abnormalities of the limbs, their origin and transmission. *Amer. Jour. Anat.* 43:167-206. 1929.

3. Danforth, C. H. Developmental anomalies in a special strain of mice. *Amer. Jour. Anat.* 45:275-287. 1930.

4. Kobozieff, N. and Pomriaskinsky-Kobozieff, N. A. Sur les anomalies congénitales et héréditaires des membres chez les souris. *Comptes Rendus de la Société de Biology* 140: 200-202. 1946.

◆

A Case of Twin Embryos of the Guinea Fowl

(*Continued from page* 176)

From their proximity, anatomical fusion and likeness of age and sex, it may be concluded that they are monozygotic, probably from two axes resulting from double gastrulation.

Literature Cited

1. Daniel, J. C. Reversal of sex chromatin patterns in birds. *Jour. Colorado-Wyoming Acad. Sci.* 4:49. 1958.

2. Frost, D. M. A case of abnormal twinning in chick embryos. *Biol. Rev. Coll. City of New York* 9: 34-37. 1947.

3. Hess, C. W., M. Juhn, and M. Jull. Two cases of twin chicks with a single umbilical cord and yolk. *Poultry Sci.* 26: 648-650. 1947.

4. Komai, T. and K. Nakamura. Types of duplicity found in chicks and duckling. *Memoirs Coll. of Sci. Kyoto Imp. Univ.* 14. 1938.

5. Nalbandov, A. A case of viable twin chicks. *Jour. Hered.* 33: 53-54. 1942.

6. Newman, H. H. Twin and triplet chick embryos. *Jour. Hered.* 31: 371-378. 1940.

7. Riddle, O. On the cause of twinning and abnormal development in birds. *Am. Jour. Anat.* 32: 199-252. 1923.

8. Sturkie, P. D. The production of twins in *Gallus domesticus*. *Jour. Exptl. Zool.* 101: 51-63. 1946.

9. Waddington, C. H. Twinning in chick embryos. *Jour. Hered.* 32: 268-270. 1941.

INFORMATION FOR CONTRIBUTORS

Editorial Policy: Membership in the American Genetic Association is not a prerequisite for publication in the JOURNAL OF HEREDITY. Papers will be judged by the editorial board on the basis of their original data, interpretation or review with a limit of eight printed pages. An article exceeding this limit will be charged at the rate of $20.00 for each additional page. Papers must be submitted solely to the JOURNAL OF HEREDITY and must not be reprinted or translated without consent of the Editor. At least six months should be allowed for publication.

Manuscripts: The entire manuscript should be typed double-space, allowing good margins. It is requested that captions and legends for figures be typed on sheets separate from the text. Footnotes are not desirable and should be avoided wherever possible. Purely descriptive matter should be incorporated into the text, and literature citations should be treated as noted below. Titles should be condensed to fifty letters or less, with a short sub-head when necessary. Manuscripts will not be returned to the author.

Illustrations: Tables and illustrations should be planned to fit the type-page (5 x 7⅝ inches). Tables should have a descriptive heading. Halftones and line drawings will be accepted in moderate numbers. Drawings must be submitted in a form suitable for cuts. Contributors will be expected to bear half of the cost for excessive numbers of half-tone illustrations. Original photographs should be submitted unmounted in the form of clear black and white prints on glossy paper. Care should be taken to see that they cannot be bent or folded in handling, and paper clips should not be used. All imperfections in the original copy are reproduced.

References: References to the literature should be cited by the author's name and literature cited reference number e.g. (Smith and Jones[3]), or by the number alone where applicable. The bibliography should be arranged alphabetically by author under the heading, *Literature Cited*. Complete reference is necessary and the arrangement should be as follows: Smith, L. C. and C. B. Jones. Inherited skin-defect in cattle. *Jour. Hered.* 32: 419-423. 1941.

Proofs and Reprints: Galley proofs will be sent to the author, and the corrected proof and reprint order should be returned to the Editor. Page proof will be sent, when time permits. One hundred reprints, without covers, are furnished free of charge to the author.

All manuscripts and correspondence should be addressed to the Managing Editor, JOURNAL OF HEREDITY, 1507 M Street, N. W., Washington 5, D. C.

The American Genetic Association

An incorporated organization devoted to promoting a knowledge of the laws of heredity and their application to the improvement of plants, animals, and human racial stocks.

Its membership is composed of men of science, teachers, publicists, physicians, clergymen, parents, students, horticulturists, and breeders of live stock throughout the world.

The Association owns the JOURNAL OF HEREDITY, which is published bi-monthly and sent to each member without additional cost. Every member is thus a part owner of the JOURNAL.

Membership imposes no burdensome obligations. The Association, which is co-operative in nature, welcomes assistance in research, but does not demand it. Members are invited to submit discussions of the results of their research, accompanied by new and unusual photographs. All papers received will be given full consideration by the editorial board.

Manuscripts should be sent to the Editorial Office of the JOURNAL, 1507 M Street, N. W., Washington 5, D. C.

Proof: In order to facilitate prompt publication only galley proof will be sent to authors.

Reprints: On request the author will receive gratis 100 reprints without covers. Additional copies will be furnished at cost.

REQUIREMENTS FOR MEMBERSHIP

Subject to the approval of the Council, any person interested in the improvement of the human race or the creation of better varieties of plants and animals, is eligible for membership.

The Association welcomes all who are interested in its program, and the Secretary will be glad to answer any inquiries.

Annual dues, giving the right to attend all meetings, and receive the JOURNAL OF HEREDITY, are $6.00 within the United States and its possessions; $6.25 in Canada, and $7.00 in all other foreign countries; life membership, $100. Subscription to the JOURNAL is $8.00 per year (foreign postage extra.)

If you are not already a member, and want to become one, or if you know of anyone who would be interested in membership, write to

THE AMERICAN GENETIC ASSOCIATION

Washington 5, D. C.